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CONTENTS

KINSELLA, T. J., MARIETTE, E. S., MATILL, P. M., FENGER, E. P. K., FUNK, V. K., LARSON, L. M., COHEN, S. S., AND NEMEC, F. C.: Thoracoplasty in the Treatment of Pulmonary Tuberculosis. An Analysis of Results Five to Twenty-six Years after Operation 113
RAUCHWELGER, SOLOMON, AND ERSKINE, FREDERICK A.: Significant Electrocardiographic Changes Following Chest Surgery 128
RIGGINS, H. MCLEOD, AND HINSHAW, H. CORWIN: Streptomycin-Tuberculosis Research Project of the American Trudeau Society. A Summary Report. 140
LURIE, MAX B., ABRAMSON, SAMUEL, AND ALLISON, MARVIN J.: Constitutional Factors in Resistance to Infection. I. The Effect of Estrogen and Chorionic Gonadotropin on the Course of Tuberculosis in Highly Inbred Rabbits. 168
LURIE, MAX B., HARRIS, T. N., ABRAMSON, SAMUEL, AND ALLISON, MARVIN J.: Constitutional Factors in Resistance to Infection. II. The Effect of Estrogen on Tuberculin Skin Sensitivity and on the Allergy of the Internal Tissues 186
LURIE, MAX B., ABRAMSON, SAMUEL, HEPPLESTON, A. G., AND ALLISON, MARVIN J.: Constitutional Factors in Resistance to Infection. III. On the Mode of Action of Estrogen and Gonadotropin on the Progress of Tuberculosis 198
LETTERS TO THE EDITORS:
Streptomycin-dependent Strains of *Mycobacterium Tuberculosis*. LENERT, T. F., AND HOBBY, G. L. 219
Tuberculostatic Activity of Aureomycin *in vitro* and *in vivo*. STEENKEN, W., JR., AND WOLINSKY, E. 221
ABSTRACTS 29

ANNUAL MEETING • DETROIT, MICHIGAN
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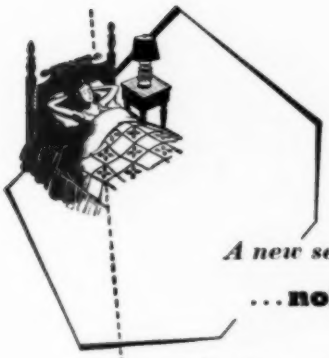

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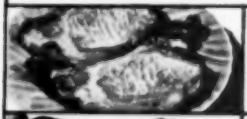
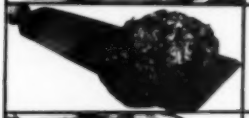
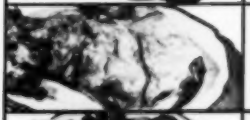
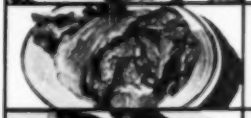
 PORK	EXCELLENT	EXCELLENT	FAIR	EXCELLENT	EXCELLENT
 BEEF	EXCELLENT	FAIR	EXCELLENT	EXCELLENT	EXCELLENT
 LAMB	EXCELLENT	FAIR	GOOD	EXCELLENT	EXCELLENT
 VEAL	EXCELLENT	GOOD	GOOD	EXCELLENT	EXCELLENT
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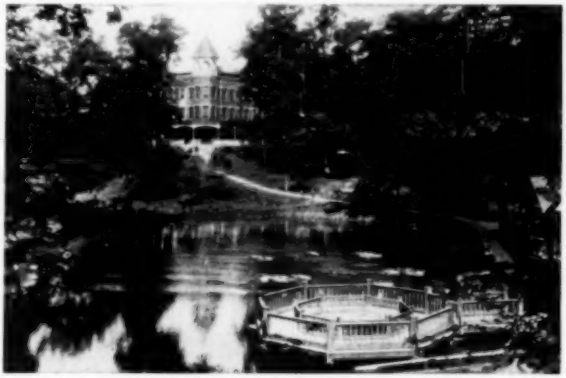

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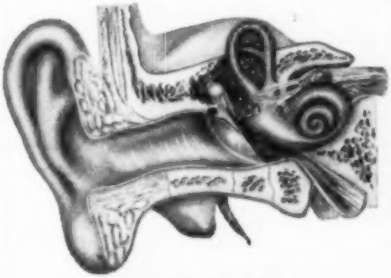

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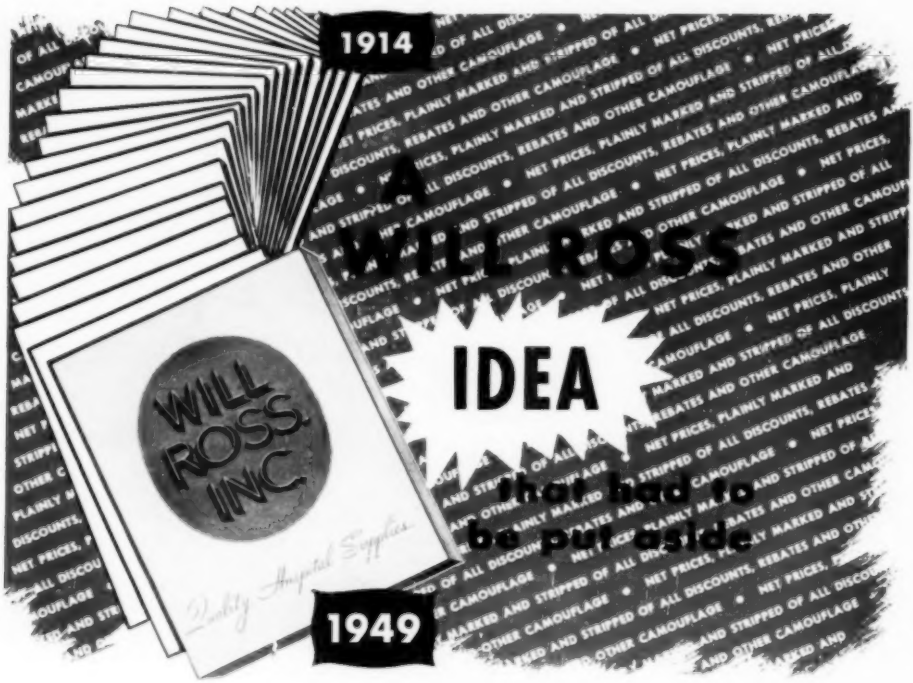

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THORACOPLASTY IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1,2]

An Analysis of Results Five to Twenty-six Years after Operation

T. J. KINSELLA, E. S. MARIETTE, P. M. MATILL, E. P. K. FENGER, V. K. FUNK, L. M. LARSON, S. S. COHEN AND F. C. NEMEC

INTRODUCTION

Extrapleural thoracoplasty has occupied a role of increasing importance over the years in the surgical treatment of pulmonary tuberculosis. The value of the procedure in situations where it is definitely indicated has never been seriously challenged and the early results obtained have been eminently satisfactory. Because of the treacherous nature of tuberculosis and the tendency for its victims to undergo relapses and exacerbations of the disease from time to time, it was felt that a careful analysis of the late results obtained in a large series of patients, carefully controlled and accurately followed for many years, might reveal much information of value in guiding the future handling of this disease.

OBSERVATIONS

Glen Lake Sanatorium, a public institution of 640 adult bed capacity, caring for the tuberculous individuals of Hennepin County, including Minneapolis, was first opened in 1916 and has been in continuous operation for the past thirty-two years. During the period of this study, 1916 through 1942, a total of 6,394 patients suffering from pulmonary tuberculosis were admitted to this institution for treatment. This group included 3,773 patients with far advanced disease, 1,978 with moderately advanced disease, and 693 with minimal tuberculosis. The senior medical and surgical staff has, with one or two exceptions, remained almost unchanged for the entire period. Thus the factors of indications, selections of patients, preoperative and postoperative care and the like, which might influence the series, have remained remarkably constant throughout except as altered by accrued experience.

The first thoracoplasty operation at Glen Lake Sanatorium was performed on January 22, 1922 by the late Dr. Arthur A. Law who pioneered through the early portion of the series. He remained the surgical director until early in 1928 and on his retirement was succeeded by one of the writers (T. J. K.). Since the fall of 1940 another two of the writers (V. K. F. and L. M. L.) have carried on the surgical work. During the study, a total of 613 patients, or 9.6 per cent of the patients with pulmonary tuberculosis admitted to the institution from 1916 to 1943, were subjected to thoracoplasty. Six hundred and nine of these patients had unilateral thoracoplasty carried out in 1,562 operations, while four had

[1] From the Glen Lake Sanatorium, Oak Terrace, Minnesota.

[2] Presented before the Medical Section, as part of the symposium on *Surgery—Trends—Late Results*, at the 44th Annual Meeting of the National Tuberculosis Association, New York, New York, June 16, 1948.

bilateral thoracoplasties performed in 12 operations, making a total of 1,574 (including 88 revisions) operations for the entire series, an average of 2.6 operations per patient (tables 1, 2, 3).

Sex, Age and Side of Operation

The series included 270 males and 343 females, a distribution which was comparable with that found in the general population of the Sanatorium. The age range for the males was from eleven to sixty-four years and for females from sixteen to sixty-six years. Eighteen of the patients were between ten and twenty years of age, 213 between twenty and thirty, 206 between thirty and forty, 121 between forty and fifty, 42 from fifty to sixty, while 13 were beyond the age of sixty at the time surgery was undertaken. In 295 of the patients the thoracoplasty was performed on the right side, in 314 the operation was on the left, and in 4 the procedure was bilateral. Twelve and one-tenth per cent of the patients

TABLE 1

Stage of disease at time of thoracoplasty, 1922 to 1943

STAGE OF DISEASE	SEX OF PATIENTS			ADMISSIONS ADULT PULMONARY 1916 TO 1943
	Male	Female	Total	
Far advanced	176	211	387	3,773
Moderately advanced	93	132	225	1,978
Minimal	1	0	1	643
Total	270	343	613	6,394

Thoracoplasty 613 = 9.6 per cent of admissions.

operated upon presented unilateral disease at the time of their surgery, while 87.9 per cent had bilateral involvement, though usually the contralateral process was not recently active or progressive. At the time of the thoracoplasty operation contralateral pneumothorax was present in 27 patients, contralateral paraffin pneumonolysis in 3, and contralateral thoracoplasty in 4.

Selection of Patients

Prospective candidates for thoracoplasty were always presented before a staff conference by the physician upon whose service the patient was in residence. At this time a complete review of the history, physical examination, laboratory findings, and all significant roentgenograms was presented, together with the physician's evaluation of the situation and recommendations as to treatment or requests for aid. In all situations the evaluation of the attending physician in closest contact with the patient was duly honored and his recommendations were given careful consideration. In general, patients with acute or recent disease were not deemed suitable for thoracoplasty, the emphasis being placed on the selection of the "good chronic" for surgical treatment, though there were many deviations from this general plan. No patient who had a reasonable chance of obtaining a satisfactory result from surgical treatment was denied

surgery because of being considered to be a "bad risk" or because of the presence of tuberculous or nontuberculous extrapulmonary complications.

TABLE 2

Total thoracoplasties, 1922 to 1943 (excluding 88 reoperations)

NUMBER OF OPERATIONS PER PATIENT	UNILATERAL THORACOPLASTY					
	Known Deaths		Others		Total	
	Patients	Operations	Patients	Operations	Patients	Operations
1 Operation	45	45	54	54	99	99
2 Operations	79	158	157	314	236	472
3 Operations	51	153	153	459	204	612
4 Operations	24	96	37	148	61	244
5 Operations	1	5	6	30	7	35
6 Operations	2	12			2	12
Total	202	469	407	1,005	609	1,474
	BILATERAL THORACOPLASTY					
1 Operation each side	1	2			1	2
1 Operation 1 side, 2 on other			2	6	2	6
1 Operation 1 side, 3 on other			1	4	1	4
Total	1	2	3	10	4	12
Grand total	203	471	410	1,015	613	1,486

TABLE 3

Type of operation and side

	WITH ANTEROLATERAL		WITHOUT ANTEROLATERAL		TOTAL
	Right	Left	Right	Left	
Complete	42	53	48	70	213
Partial	76	69	129	122	396
Total	118	122	177	192	609

Exclusive of 4 with bilateral

Indications

By far the greatest number of patients subjected to thoracoplasty over the years have been operated upon because of the presence of pulmonary cavitation,

either large or small, and because of the persistence of phenomena arising from the cavity, such as continued cough with discharge of tubercle bacilli, or pulmonary hemorrhage. A smaller group of patients were operated on because of primary pyothorax or pyopneumothorax complicating previous attempts to control the disease by artificial pneumothorax. A few patients were subjected to extrapleural thoracoplasty for other indications, *e.g.*, the obliteration of an extrapleural empyema pocket, to intensify the collapse of a previously unsuccessful extrapleural pneumolysis, to relieve traction, displacement or discomfort produced by the atelectatic retracting of an upper lobe tuberculosis, or to relieve bronchial kinking. In the majority of the patients operated upon attempts at the induction of artificial pneumothorax had either failed or the treatment had proved unsatisfactory. A few primary thoracoplasties without previous attempt at pneumothorax were carried out even many years ago under special circumstances.

Three hundred and eighty-three of the patients (172 males and 211 females) were operated upon because of far advanced disease, 229 (97 males and 132 females) because of moderately advanced disease, and one male had a complete thoracoplasty because of a minimal tuberculous lesion with pulmonary hemorrhage. The stage of disease mentioned indicates the stage of disease at the time the surgery was undertaken, not at the time of admission to the sanatorium and has been made according to the present National Tuberculosis Association classification of disease as evidenced on the preoperative roentgenograms. These films were completely re-examined by the writers in the preparation of this paper. Such a reclassification has been necessitated by the changes of classification which have been made on two or three occasions since the inception of this series.

Type of Operation

The earlier portion of this series was represented by a group of patients in whom the Wilms-Sauerbruch type of limited posterior paravertebral resection was carried out. Almost invariably the lower stage procedure was done first if multiple stage operations were carried out. There was but one complete single stage operation in the series and the patient died within twenty-four hours. It was considered mandatory at this time to perform a phrenic nerve interruption preliminary to thoracoplasty. As the inadequacy of the limited resections in patients with extensive disease gradually became evident, wider and wider segments of rib were removed until the operation approached the Brauer subscapular type of thoracoplasty. By the spring of 1927 a change was made so that an upper posterior thoracoplasty was usually done first and the complete posterior thoracoplasty divided into three stages instead of two. Anterolateral thoracoplasty was used as early as 1926 and adopted more frequently after Welles' original article on anterolateral thoracoplasty was published in 1927 (2). At first it was used almost invariably as a sequence to complete posterior thoracoplasty. Later it was combined with a partial thoracoplasty and soon came to be used as the second stage of the series with the upper posterior first and the intermediate posterior third. The use of the lateral or anterolateral operation as a first stage was not deemed advisable and, therefore, not used in this series.

The interval between operations in the earlier portion of the series was excessively long, at times even a matter of several months. Gradually, however, as the series developed, an interval of approximately three weeks between stages was adopted and was utilized in the majority of the cases. More recently this interval has been reduced to two weeks.

All of the earlier thoracoplasties were carried out in an extremely rapid time "with one eye on the clock" in an attempt to complete the operation before shock supervened. Records as low as nine minutes from incision to last stitch of closure for a lower stage six rib thoracoplasty, and eleven minutes from incision to last stitch of closure for an upper stage five rib thoracoplasty were made. Needless to say, excessive blood loss, long residual vertebral rib stumps, perforation of the

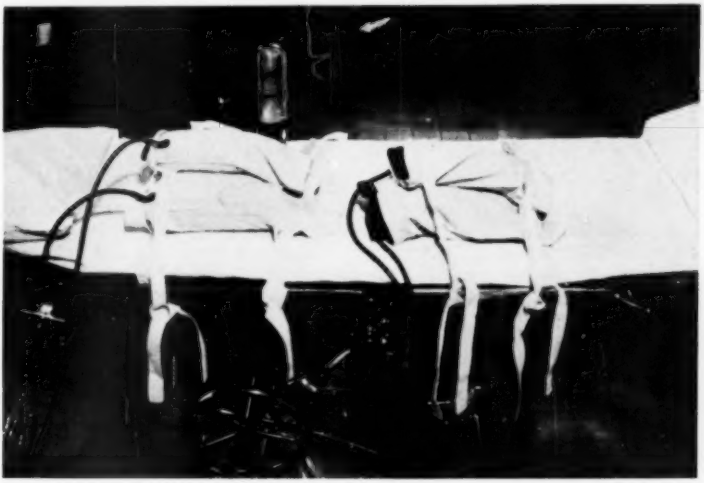

FIG. 1. Balloons used in posturing patient on the operating table.

pleura, and severe shock were frequently encountered. With improvements in technique and particularly with a change to local infiltration as the anesthesia of choice in 1929, the emphasis on speed was discarded and a more careful, meticulous type of thoracoplasty carried out, resulting in improved postoperative condition of the patients. While some of the earlier operations were carried out in the direct lateral position, a change was soon made to a three-quarters prone position which has been used consistently ever since. This position may be seen in figures 1 and 2, which illustrate the balloons used in posturing the patient and the patient in operative position upon the table.

Blood loss in the earlier thoracoplasty operations was not calculated, but is known to have been excessive. Calculations of the average blood loss for nearly 100 thoracoplasty stages carried out under local anesthesia were reported in 1937 (1) as follows: upper posterior thoracoplasty three to four ribs, 450 cc. blood loss; intermediate posterior thoracoplasty three to four ribs, 296 cc.; lower

posterior thoracoplasty three to four ribs, 196 cc.; and anterolateral thoracoplasty three to four ribs and cartilages, 250 cc. These values were obtained by calculation of the hemoglobin content of all of the sponges and packs used during the course of the operation and all of the dressings used by the patients in the first forty-eight hours after surgery and the result was then expressed in terms of cubic centimeters of blood of a hemoglobin content at the patient's preoperative hemoglobin level.

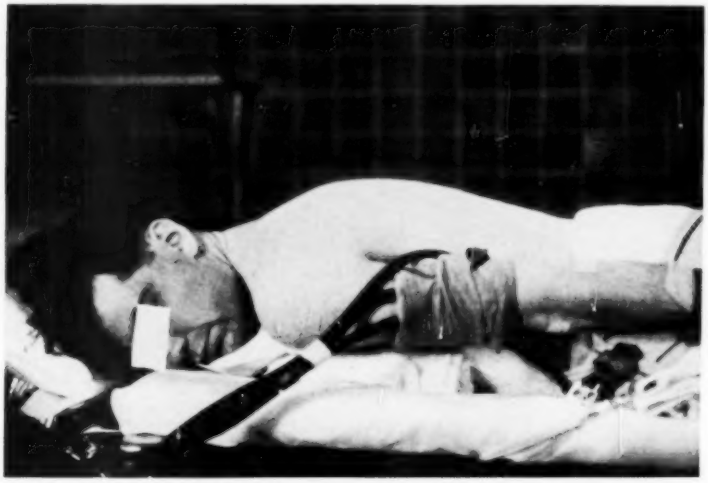

FIG. 2. Patient in operative position.

Preoperative Preparation

The preoperative preparation of the patient was usually as simple as possible in an effort to disturb the patient's normal routine to a minimal degree. A careful survey of all factors was always made so that the risk might be evaluated and all necessary precautions taken to protect the patient in every possible way. Subsequent to 1926, the cardiovascular status of all prospective thoracoplasty patients was carefully studied by the attending cardiologist, Dr. Olga S. Hansen, and electrocardiographic studies were made before operation, between stages, and thereafter. A special effort was always made to ensure that the patient had raised his usual quota of sputum before surgery was undertaken. For this reason for many years the majority of the thoracoplasties were carried out in the afternoon when the patient has usually completed his daily cavity drainage.

If the operation was to be carried out under local anesthesia, the patient was given 0.1 Gm. of nembutal at two hours and one hour before surgery, followed by a hypodermic of 0.010 Gm. of morphine or pantapon at approximately one-half hour before going to the operating room. An additional hypodermic of 0.060 Gm. of codeine was sent to the operating room with the patient to be used at any time when unusual apprehension, nervousness or other symptoms might appear during the course of the surgery.

The original operations in the series were done entirely under nitrous oxide anesthesia, but very soon paravertebral block with novocain followed by nitrous oxide anesthesia became the routine anesthetic. Through 1928 and 1929 occasional cases were done under local anesthesia alone. With increasing experience, local anesthesia became the anesthetic of choice and thereafter was used almost exclusively for operations which could be carried on under this type of anesthesia alone. In no instance was it necessary to change to a general anesthesia when this sequence had not been anticipated before surgery was undertaken. Nitrous oxide, ethylene, or cyclopropane were administered in addition to the local anesthesia in a few instances. The general impression that under local infiltra-

FIG. 3. Support of chest while patient is encouraged to cough.

tion anesthesia the patients came through their surgery in better condition with less shock, less blood loss and in better general condition has been adequately confirmed over the years. The ability of the patient to cough and expectorate during, as well as immediately following, surgery has been a distinct advantage, particularly in the patient with larger amounts of sputum.

Postoperative Care

Postoperatively there are a number of points which were considered important to the safety and comfort of the patient. In order to avoid aspiration of material into the good lung, care was taken never to turn the patient or allow the patient to turn so that the uninvolved lung was dependent while awake or asleep. Opiates were used in dosage sufficient to control distress but not to destroy the cough reflex. The patient was required to cough and expectorate sputum at intervals from the time of surgery, using expectorants and proper support to the deribbed chest wall, as illustrated in figure 3. Fluids were routinely administered

postoperatively by retention enema and by the intravenous or subcutaneous routes when necessary. Transfusions were used on but few occasions in the whole series. Patients were permitted and encouraged to take fluids and food by mouth as soon as tolerated. Many patients were able to take a reasonably normal meal within a few hours after the operation.

If any paradoxical chest wall motion was noted, sandbags were immediately applied to the area or if necessary the chest was firmly strapped with adhesive tape over rubber sponges. Local area dressings were changed as necessary. Penrose drain tubes which were used routinely were removed in approximately forty-eight hours. Patients were encouraged and urged to move around as soon as possible after surgery. They were routinely required to sit up for dressings the morning following the operation and subsequently at frequent intervals. Early motion of the arm and shoulder on the operated side was insisted upon and every attempt was made to keep the patient from feeling that the operation would in any way cripple him. Because surgery alone does not cure tuberculosis, all patients were maintained on strict bed-rest for a period of at least six months following the completion of all stages of the thoracoplasty procedure.

Mortality

Twenty-two of the 613 patients in the entire series died within fourteen days of the operation (table 4). This represents a mortality rate of 3.59 per cent per patient and 1.40 per cent per operation. A total of 34 patients died within eight weeks of the time of operation, yielding an eight weeks' mortality of 5.55 per cent per patient or 2.16 per cent per operation (Table 5). One patient died upon the operating table and two others died during the first twenty-four hours following surgery, apparently from shock. Three died on the second day following operation, one on the third, 2 on the fourth, 3 on the fifth, 2 on the seventh, one on the eighth, 2 on the ninth, 2 on the tenth, and 2 on the eleventh day after surgery. Eleven of the deaths may justly be attributed to an extension of the tuberculosis. Two of the patients definitely aspirated material through a bronchial fistula to cause the spread of the disease. An additional patient, considered at postmortem examination as having died of asphyxia, probably also should have been classified as a tuberculous death. Two, who on the death certificates were listed as pneumonia, were proven at postmortem examination to have nontuberculous pneumonias. One patient was listed as dying of pulmonary edema, one from right heart failure and one on postmortem examination as tuberculous myocarditis and rheumatic endocarditis, making a total of 3 patients apparently dying of cardiac conditions. One died from a cerebral vascular accident, probably resulting from low blood pressure and thrombosis, one from a pulmonary embolism on the eighth postoperative day, and one patient from an accidental pneumothorax produced on the fourth postoperative day by an attempt to refill a contralateral pneumothorax which was present at the time of surgery. The cause of death in 15 of this group was established at necropsy.

Eleven patients died after the first stage operation, 6 after the second, 4 after the third, and one after the fourth operation. Twelve deaths occurred after

TABLE 4

Thoracoplasty series mortality, 1922 to 1943

DEATH SUBSEQUENT TO OPERATION	
8 weeks	34
8 weeks to 4 years (inclusive)	92
5 to 9 years	55
10 to 14 years	17
15 to 19 years	5
Total	203
SURVIVAL AFTER LAST OPERATION (ANALYSIS MARCH 1948)	
1 to 4 years	8
5 to 9 years	127
10 to 14 years	99
15 to 19 years	60
20 years and over	20
Total	314
SURVIVAL AFTER LAST OPERATION (RECENT INFORMATION UNOBTAINABLE MARCH 1948)	
1 to 4 years	43
5 to 9 years	38
10 to 14 years	12
15 to 19 years	3
Total	96

TABLE 5

Thoracoplasty series mortality, 1922 to 1943

OPERATIVE DEATHS	PER CENT OF PATIENTS	PER CENT OF OPERATIONS
2 Weeks	3.60	1.40
2 Months	5.55	2.16
CONDITION AT TIME OF LAST FOLLOW-UP		
Alive	410	66.88
Dead from all causes	203	33.12
Unrelated Cause	34	5.55
Tuberculosis	169	27.57

local anesthesia, 7 after nitrous oxide and local, 2 after ethylene and one following spinal anesthesia. There were 11 males and 11 females. The age groups were quite representative of the age groups in the whole series, with only 2 of the deaths occurring in patients over 45 years of age, and 2 under the age of 20.

An additional 12 patients died of the following causes within six weeks or two months of operation: 8 from pulmonary tuberculosis; one from tuberculous meningitis; one from pulmonary embolism; one from chest wall flutter; and one from cardiovascular failure with peripheral edema. One of the tuberculous deaths in this group also resulted from aspiration of pus from a pyopneumothorax cavity through a bronchial fistula. The cause of death in 7 of this group was confirmed by postmortem studies.

A graphic representation of how the operative mortality has decreased with the development of the series may be seen in figure 4. Each vertical column in the figure represents the number of operations (and the number of patients sub-

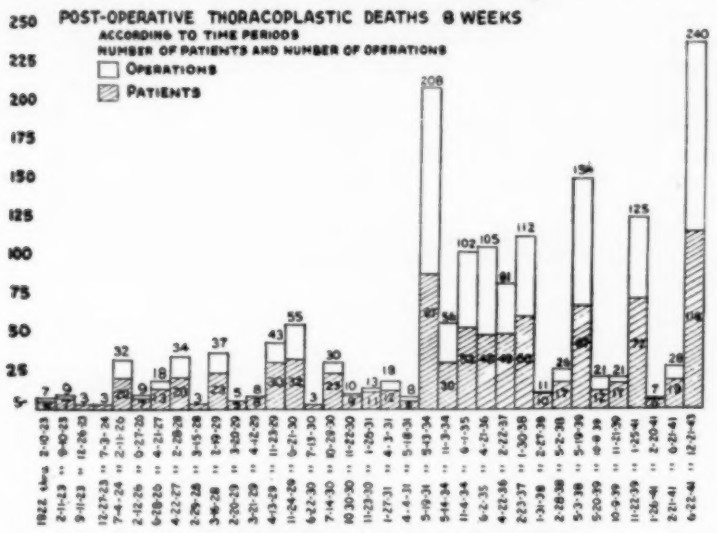

FIG. 4. Postoperative thoracoplastic deaths 8 weeks.

jected to surgery) which were performed during the various periods of time which elapsed between any two postoperative deaths. All deaths, regardless of cause, which occurred during the eight week period following operation are included. The death which interrupted the series on May 13, 1934, after 208 consecutive operations on 87 patients, was caused by an accidental pneumothorax on the contralateral side induced in an attempt to refill a pneumothorax pocket present before operation. The operative mortality (within eight weeks) since 1931 has been 3.0 per cent per patient and 1.16 per cent per operation. It is interesting to note that 59 per cent of the mortality within eight weeks has occurred in the first 21 per cent of the series.

Shock was a very prominent feature in all of the earlier operations and was frequently of severe degree. It is very difficult from the study of charts alone to obtain any accurate indications of the frequency of this condition because of the

great variation in its degree and in the interpretation of what constitutes shock. Three patients definitely died in this state. In the latter portion of the series shock became a factor of much less importance and in a severe form was rarely, if ever, seen.

Blood transfusions were rarely used in this series which was carried out before the days of blood banks with blood freely available. It is quite probable that in the early portion of the series especially the use of blood transfusions would have been a distinct advantage. They have not been necessary in the latter portion of the series, but might definitely have hastened the recovery of some patients and possibly offered a chance for a shorter interval between stages than the one customarily used.

Major wound infections were not of frequent occurrence and records of only 35 or 2.2 per cent can be found listed. Perforation of the pleura was noted not infrequently in the early portion of the series where an extremely rapid surgical resection was carried out. In the latter portion of the series, however, with a more careful operative technique, perforations have rarely occurred. Tuberculous sinuses resulting from damage to lung, pleura or to tuberculous lymph nodes have been recorded in only 6 patients in the whole series.

Scoliosis of any degree whatsoever as noted on the roentgenogram has been reported in 165 patients or 26.9 per cent of the entire series, but in only 13 (2.12 per cent) was it either moderate or marked. It was seen most frequently in young people, particularly in young girls of poor musculature. Scoliosis can be produced, however, by destruction of portions of the erector spinae group of muscles or their nerve supply, or by resection of transverse processes which represent the only remaining site of muscle attachment after removal of the ribs. No planned program for prevention and treatment of scoliosis was used in this series as it was deemed unnecessary because of the low incidence and slight degree of this complication.

Spread of Disease

A spread or exacerbation of tuberculosis was encountered 94 times in 90 patients in this series which represents a rate of 14.66 per cent per patient or 6.34 per cent per operation. Thirty-four "spreads" occurred in areas not previously involved with tuberculosis, while 60 occurred in areas in which there had previously been tuberculous disease. The latter group raises the question of how much of the total figure represents exacerbation of previously existing disease. This question cannot be definitely answered and it is probable that most of the new lesions represent a bronchiogenic spread of disease. In only 10 of the patients was the "spread" on the same side as the thoracoplasty. It is noteworthy that one-half of the patients who developed an extension of disease on the same side had a paralyzed diaphragm on that side at the time of surgery.

Thirty-eight of the patients who developed extension of disease were operated upon for large cavities, 29 for small cavities, 17 for pyopneumothorax, and 6 for miscellaneous causes. It is interesting to note that 5 of the patients with pyopneumothorax who developed a spread of disease had a definite bronchial fistula

present at the time of surgery. The hazard of a bronchial fistula in the patient with a pyopneumothorax cannot be too strongly emphasized. Sixty-two of these patients were operated upon under local anesthesia, 21 under gas and local infiltration, 3 under ethylene, one under nitrous oxide alone, one under spinal anesthesia, and one under ethylene and local anesthesia, and one under an anesthetic of unstated type. The incidence of "spread" after local anesthesia was 4.9 per cent, while under general anesthesia it was 8.4 per cent. Many other factors besides anesthetic alone must be considered in the development of a bronchiogenic spread of tuberculosis following thoracoplasty. Important among these are medications before and after operation, the ability to expectorate, the cooperation of the patient, his position in moving, and his position in bed. Consequently any correlation of "spread" to anesthesia is not an exact one. A spread of disease occurred after the first operation in 50 instances, after the second in 24, after the third in 16, after the fourth in 3 and once after the fifth operation. Among the 90 patients who developed a spread of tuberculosis following surgery, 11 died within the first two weeks after surgery, and 7 within the subsequent six weeks. Thus a total of 18 or 20 per cent died within two months. Eleven more patients died within the first year, 10 within the second year, and 19 at times varying from three to thirteen years after surgery. The spread of tuberculosis postoperatively was definitely a causative factor in the subsequent death of the majority of these individuals who died within two years.

RESULTS

Four hundred and ten, or 66.8 per cent of the entire series operated upon, still survive for a period of five to twenty-six years following surgery, giving a series mortality for all causes of 33.12 per cent. If the 34 patients who died at various times up to eighteen years following surgery from causes totally unrelated to tuberculosis are deducted from the total number of patients now dead, it may be seen that 27.57 per cent of the entire group of 613 thoracoplasty patients eventually died, either early or late, from tuberculosis or its complications. Among the causes of death unrelated to tuberculosis are: carcinoma, 9 patients; suicide, 6; accidents, 5; nontuberculous appendicitis and peritonitis, 3; cardiovascular complications, 3; psychosis, 2; nontuberculous pneumonia, 2; mesenteric thrombosis, one; meningococcic meningitis, one; nephritis following scarlet fever, one; and operation for a nontuberculous condition, one. As near as can be determined, the tuberculosis was under control at the time of death in all of these patients. These survival figures and the accompanying tables clearly indicate the stability of these lesions in these individuals once the tuberculosis has been controlled by means of extrapleural thoracoplasty.

Cavity Closure and Reversal of Infectiousness

In 88.1 per cent of the patients whose sputum contained tubercle bacilli prior to surgery, the organisms subsequently disappeared from the sputum, according to the direct examination technique used at the sanatorium over the years covered by the study. Undoubtedly, cultures of sputum or cultures of gastric washings

or guinea pig inoculations would alter this percentage materially but, as these procedures were not in use consistently over the years, they were not considered in this study where a uniform listing was desirable. As may be seen in table 6, of the patients who presented large cavities (more than 4 cm. in any one diameter prior to surgery), closure was obtained in only 49.6 per cent, with disappearance of tubercle bacilli from the sputum in 76.5 per cent. In the patients with small cavities (table 7), the incidence of cavity closure was considerably greater, namely

TABLE 6

Cavity closure and "sputum conversion"
(Large cavities)

	TOTAL	SPUTUM			
		Positive Before		Negative Before	
		Negative after	Positive after	Negative after	Positive after
Closed	70	58	8	4	0
Open	71	43	23	5	0
Total	141	101	31	9	0

49.6 per cent cavity closure.
76.5 per cent "sputum conversion."

TABLE 7

Cavity closure and "sputum conversion"
(Small cavities)

	TOTAL	SPUTUM			
		Positive Before		Negative Before	
		Negative after	Positive after	Negative after	Positive after
Closed	218	136	4	77	1
Open	99	55	15	28	1
Total	317	191	19	105	2

68.7 per cent cavity closure.
91.0 per cent "sputum conversion."

68.7 per cent, with "sputum conversion" in 90.95 per cent. Further analysis of the late results of the patients operated upon because of large and small cavity, with particular reference to mortality, both early and late, in relation to cavity closure and "sputum conversion" is not complete and will be published at a later date.

Pyopneumothorax

Ninety-four patients were operated upon because of pyopneumothorax. In this group, obliteration of the empyema pocket was obtained in 61 and question-

able closure in one additional patient, or 67.3 per cent of the patients operated upon for this condition. Failure of closure occurred in 32.7 per cent of those who survived the surgery. In this entire group, 5 died within two weeks with empyema pocket unclosed, one under eight weeks with the empyema pocket unclosed, and 6 additional patients within one year, 2 with the empyema pockets obliterated and 4 unobliterated. Twenty-five additional patients of this series died after periods longer than one year following the surgery, and of these 25, obliteration of the empyema pocket had failed to occur in 11, while 13 such cavities were definitely closed and one questionably closed at the time of death. Fifty-nine and seven-tenths per cent of the entire group still survive. The presence of a bronchial fistula in this group proved a distinct hazard for 5 of the group of 17 developed a contralateral spread of disease following surgery.

Supplementary operations to the standard thoracoplasty were frequently necessary in this series of empyemas, a complication which as a rule demands a much more extensive thoracoplasty and more numerous stages than uncomplicated pulmonary tuberculosis. Catheter drainage, rib resection for open drainage, unroofing operations, and plastic closures were all utilized in various patients in this group in an attempt to bring about obliteration of the pleural cavity. As many as seven or eight separate operations were required in some patients. Increased experience in handling empyemas has produced better results. Catheter drainage at the base laterally has been considered mandatory in all patients with definite or suspected bronchial fistulae or secondary infection. Moreover, with chemotherapeutic agents available, catheter drainage has been used with increasing frequency and to advantage in patients with straight tuberculous pyonpeumothorax without secondary infection. Closed drainage with or without irrigation or continuous suction has been of distinct value in removing all fluid from the pocket and reducing its size and bringing about more rapid obliteration of the pleural space during the course of the collapse procedures. A more complete report of this group will be made in a future communication.

Revised Operations

A series of 63 patients have been subjected to various types of supplementary and revision operations with and without packs in attempts to improve the results when the primary surgical procedures had not been entirely satisfactory. In general, these procedures were well tolerated and aided materially in improving the clinical result. Cavity closure occurred in 56.6 per cent and "sputum conversion" occurred in 73 per cent of this group of 63 patients.

SUMMARY

1. Thoracoplasty was performed on 613 tuberculous patients (1,474 individual operations) during the twenty year period 1922 to 1943.

2. Sixty-six and eight-tenths per cent of the patients are alive five to twenty-six years after operation; 27.57 per cent ultimately died of tuberculosis or its complications.

3. The operative mortalities were 3.59 per cent per patient (1.4 per cent per

operation) during the two weeks after surgery. During the eight weeks after operation the mortality was 5.55 per cent per patient and 1.16 per cent per operation.

4. Since 1931 the postoperative mortality (eight weeks) has been 3 per cent per patient and 1.16 per cent per operation.

SUMARIO

La Toracoplastia en el Tratamiento de la Tuberculos Pulmonar: Análisis de los Resultados de Cinco a Veintiséis Años después de la Operación

1. En el veintenio 1922–1943 se ejecutó la toracoplastia en 613 tuberculosos (1,574 distintas operaciones).

2. De estos enfermos, 66.8 por ciento se hallan vivos de cinco a veintiséis años después de la operación; 27.57 por ciento murieron por fin de tuberculosis o de las complicaciones de la misma.

3. La mortalidad operatoria representó 3.59 por cien enfermos (1.4 por cien operaciones) en las dos semanas consecutivas a la intervención. Durante las ocho semanas consecutivas a la intervención la mortalidad se elevó a 5.55 por cien enfermos y 1.16 por cien o operaciones.

4. Desde 1931 la mortalidad postoperatoria (ocho semanas) ha sido de 3.0 por ciento en enfermos y de 1.16 por ciento en operaciones.

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SIGNIFICANT ELECTROCARDIOGRAPHIC CHANGES FOLLOWING CHEST SURGERY[1, 2]

Electrocardiographic Changes Currently Considered Indicative of Serious Heart Disease Occurring Postoperatively in Patients with Complete Absence of Clinical Manifestations of Heart Disease

SOLOMON M. RAUCHWERGER AND FREDERICK A. ERSKINE

INTRODUCTION

The limits of normal electrocardiographic patterns have not been completely defined as yet. During World War II many studies were made at induction centers and military installations by competent investigators and numerous new variations of the normal pattern were reported (1). On the basis of this fact alone, it has been emphasized repeatedly that a diagnosis of myocardial damage should not be made solely on the basis of electrocardiographic findings.

A correlation study of the electrocardiographic findings and subsequent clinical course of 112 patients who were subjected to chest surgery has been undertaken. The surgical procedures were performed during a five year period from 1943 to 1948 at a 1,000-bed tuberculosis sanatorium and electrocardiograms were obtained before and after operation in all of the patients studied. It was noted early in the study that T-wave changes in Lead I and Lead IV, usually interpreted as signifying the presence of severe myocardial damage (3, 8, 9), occurred with notable frequency without subsequent clinical evidence of heart disease.

Because of this significant feature and also because there is relatively little information in the literature concerning electrocardiographic changes following thoracoplasty and other forms of chest surgery, the findings are reported. The benign course followed by these patients and the amount of additional surgery well tolerated after the T-wave changes occurred suggest that a revision of the electrocardiographic criteria for inoperability may be in order. Three patients included in this series did develop the electrocardiographic and clinical findings characteristic of well-known, specific disease states and these cases are discussed in detail below.

METHODS AND MATERIALS

The patients comprising this series, with the exception of 4 with bronchiectasis and 2 with pulmonary abscess, all had pulmonary tuberculosis for which surgery had been performed. There were 93 thoracoplasties, 42 on the left side and 51 on the right; 9 pneumonectomies, 3 primary and 6 secondary to a thoracoplasty; and 12 lobectomies, 8 primary and 4 secondary to a thoracoplasty. The excision cases combined with a thoracoplasty were considered as thoracoplasties in tabu-

[1] From the Department of Medicine and Surgery, Veterans Administration Oteen, North Carolina.

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TABLE 1

Table of T-wave changes following various forms of chest surgery

TYPE OF COLLAPSE	NUMBER OF PATIENTS	T_1 (I)	T_1 (L)	T_1 (D)	T_2 (I)	T_2 (L)	T_2 (D)	T_3 (I)	T_3 (L)	T_3 (D)	T_4 (I)	T_4 (L)	T_4 (D)	TOTAL NUMBER OF CHANGES
Thoracoplasty right	51	0	8	2	0	5	0	5	3	4	2	2	1	32
Thoracoplasty left	42	7	12	6	2	5	1	4	2	2	6	2	5	54
Lobectomy	8	0	2	0	0	0	0	1	0	2	1	1	1	8
Pneumonectomy	3	1	1	2	0	0	0	1	0	0	1	2	1	9
Lucite pack	8	0	3	1	0	1	0	4	1	1	0	0	1	12

(I) = inversion.
(L) = low or isoelectric.
(D) = diphasicity.

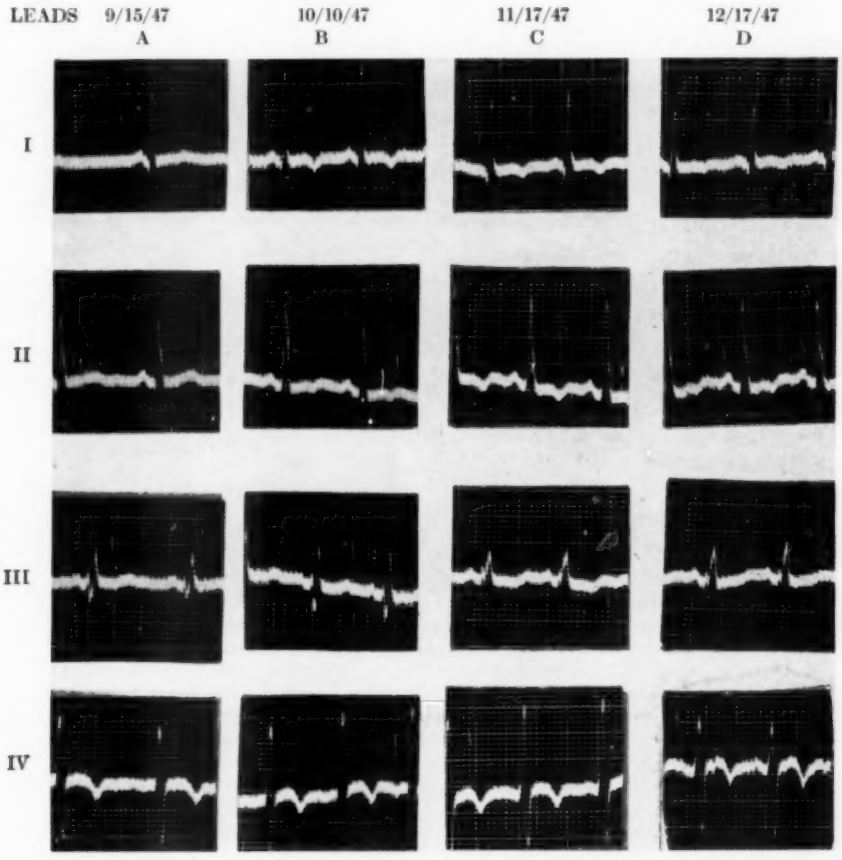

FIG. 1. Case 1 (Age 26). Roentgenogram revealed bilateral pulmonary tuberculosis with cavitation on the left. Previous collapse therapy consisted of a left pneumothorax, left phreniclasia, and pneumoperitoneum. A three-stage left thoracoplasty was performed between September 22 and December 3, 1947. Cyclopropane and ether were the anesthetic agents employed. Blood pressure was labile ranging from 110/80 to 140/100. The electrocardiographic pattern observed occurred following the first-stage thoracoplasty.

(A) Before thoracoplasty—T_4 negative; (B) after first stage—negative T_1; diphasic T_2; negative T_4; (C) before third stage—negative T_1; diphasic T_2, T_3; negative T_4. (D) After completion of thoracoplasty—negative, low T_1; negative T_4.

lating results as no significant data resulted from further breakdown. There were 8 cases of extrapleural pneumonolysis with plombage (methyl methacrylate), 3 alone, and 5 combined in some form with a thoracoplasty.

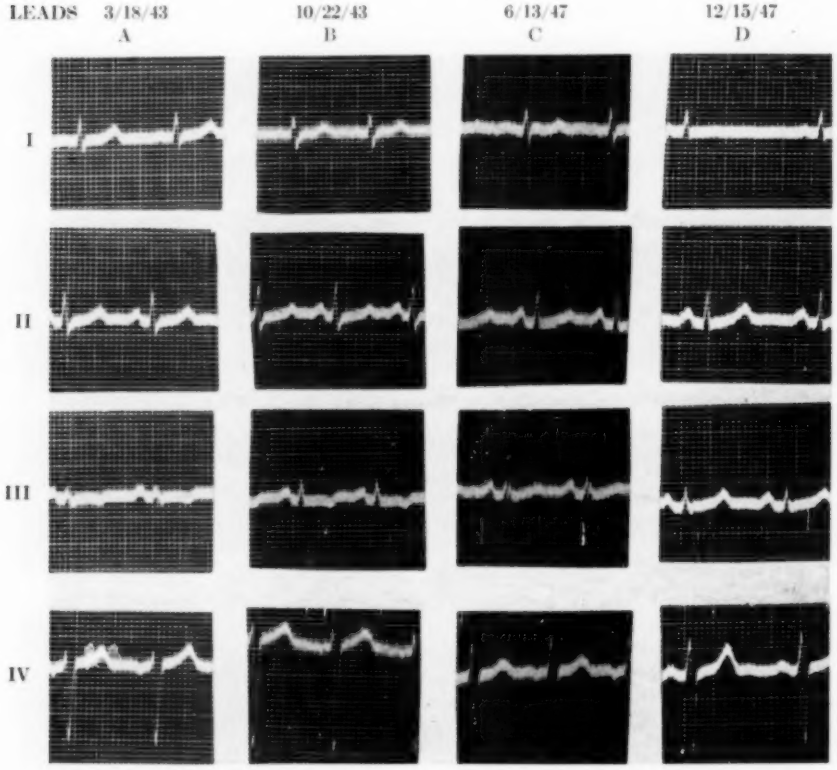

FIG. 2. Case 2 (Age 55). Chest roentgenogram revealed extensive fibro-exudative disease with cavitation on the left and a fibrotic lesion on the right. This patient had a three-stage left thoracoplasty including a revision thoracoplasty performed between November 5, 1943, and September 22, 1944. On June 17, 1947, a left pneumonectomy was done. Again cyclopropane and ether were the anesthetic agents. The electrocardiographic changes seen in tracing dated December 15, 1947 were first noted June 20, 1947 but the tracing for this date could not be located.

(A) Before surgery—normal tracing; (D) Following pneumonectomy—T_1 isoelectric.

Each patient had a complete cardiac examination, including an electrocardiogram, before and after operations. The electrocardiographic study included the four standard leads (I, II, III, and CF IV) in all cases, and in the doubtful cases the six classical chest leads were also employed. In addition, an electrocardiogram was made prior to each stage of thoracoplasty. The examinations also

included a clinical evaluation of the patient on the morning surgery was to be performed.

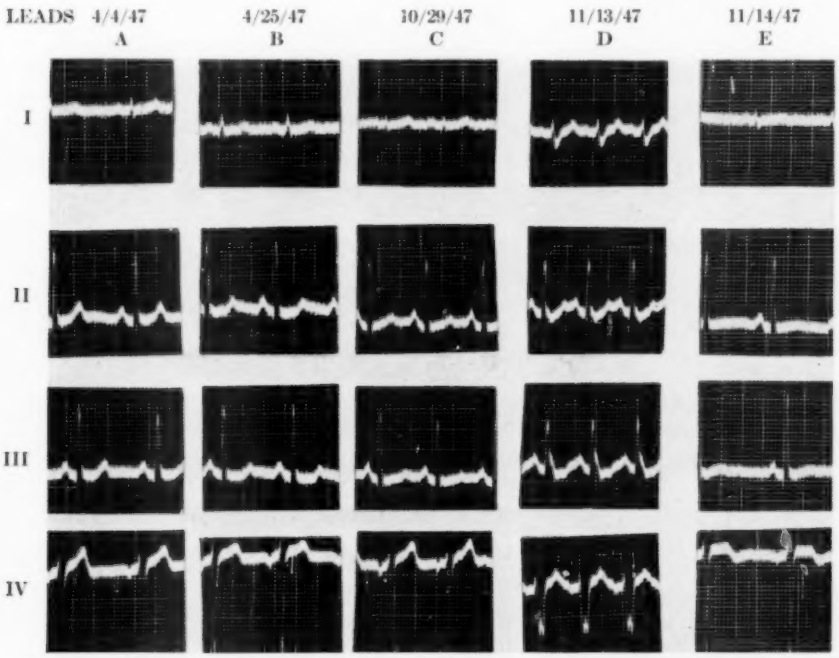

FIG. 3. Case 3 (Age 52). Patient was admitted with a diagnosis of far advanced pulmonary tuberculosis with cavitation on the right. A three-stage right thoracoplasty was done between April 7 and May 13, 1947. On November 12, 1947, he had an excision of an osteomyelitic rib. Approximately twenty-four hours later, he developed signs and symptoms of a pulmonary embolism on the left side, manifested by sudden onset of severe pain in the chest, more marked on the left, and accompanied by evidence of shock. Anticoagulants and supportive measures were administered and recovery was uneventful. An electrocardiogram on November 13, 1947, revealed the findings characteristic of pulmonary embolism. The electrocardiographic changes gradually resolved over the ensuing several days.

(A) Before surgery—normal pattern; (B), (C) following thoracoplasty—no change; (D) after excision of osteomylitic rib—deep S_1; depressed ST_2, ST_3; (E) 24 hours after (D)—low, slurred QRS_1 and poor T-waves.

RESULTS

Pronounced T-wave changes in Leads I and IV were noted in 34 of the 112 cases reviewed. In 18 there was marked lowering (to less than 100 microvolts) or inversion of T_1 alone. In 16 cases there occurred a combination of lowered or inverted T_1 with a similar change in T_4. The T-wave changes in Leads II and III were as tabulated in table 1.

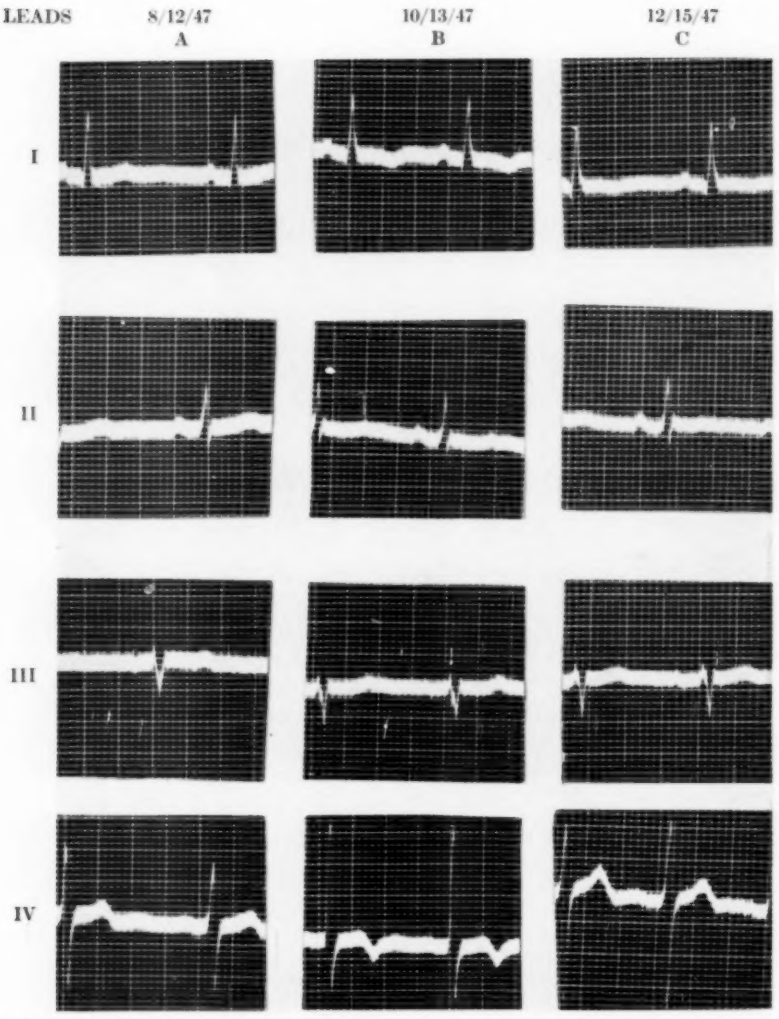

FIG. 4. Case 4 (Age 24). Roentgenogram revealed a bilateral productive lesion with some degree of atelectasis of the left lung. A two-stage left thoracoplasty was performed between September 15 and October 20, 1947. Cyclopropane and ether were the anesthetic agents used. Changes in the electrocardiographic pattern occurred after the first-stage thoracoplasty.

(A) Before surgery—left axis deviation; (B) following first stage—inverted T_1 and T_4; (C) after completion of thoracoplasty—low, inverted T_1; T_4 now upright.

Other changes occurring less frequently (not statistically significant) were: (a) tendency for increase in amplitude of P-waves *without* evidence of right heart strain in the vast majority of instances; (b) elevation in S-T segment in 4 cases, in Leads II, III and IV; (c) frequent extrasystoles (especially prominent following 2 pneumonectomies); and (d) occasional splitting of QRS_4. In review of the records of 23 patients who had a phrenemphraxis with or without pneumoperitoneum or pneumothorax, the only unusual findings were inversion of T_4 in two (table 1), and inversion of T_1 in one.

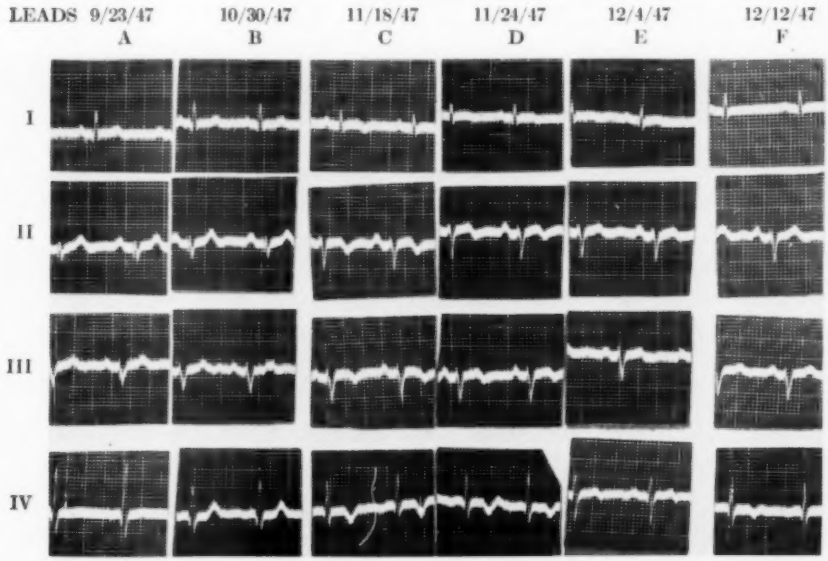

FIG. 5. Case 5 (Age 46). Roentgenogram revealed bilateral pulmonary tuberculosis of a fibro-caseous nature with cavitation on the right. Previous collapse therapy consisted of an unsuccessful right pneumothorax, a right phreniclasia, and pneumoperitoneum. On October 17, 1947, an anterior first-stage right thoracoplasty, and on October 31, 1947, a posterior right thoracoplasty were performed. On November16, 1947, the patient experienced an acute episode of pain in the left side of the chest which gradually increased in intensity, especially on deep inspiration. An electrocardiographic tracing taken on Novebmer 18, 1947, revealed a pattern compatible with that of an acute pericarditis. Frequent examinations failed to reveal evidence of a friction rub or a pericardial effusion. Subsequent tracings showed resolution of the acute process and on December 16, 1947, a second-stage posterior thoracoplasty was performed under cyclopropane and ether. Between January 16 and February 2, 1948, he had a two-stage cavernostomy with no untoward effect. Serial electrocardiograms up through February 4, 1948, revealed normal findings.

(A) Before surgery—left axis deviation; (B) before second stage thoracoplasty—no change; (C) T-waves inverted in all leads; (D) six days after (C)—T waves diphasic; (E) T_2, T_3 now upright; T_1 and T_4 low and diphasic; (F) T_1 isoelectric; T_4 inverted.

The presence of a negative T-wave in Lead I, especially in conjunction with a

negative or diphasic T-wave in Lead IV (figure 1), if persistent, is currently considered strongly suggestive of myocardial damage (3, 8, 9). Alterations in Leads II, III, and CF IV alone may be produced by shifting of the mediastinal contents without coexistent heart damage (figure 3). This is equally true

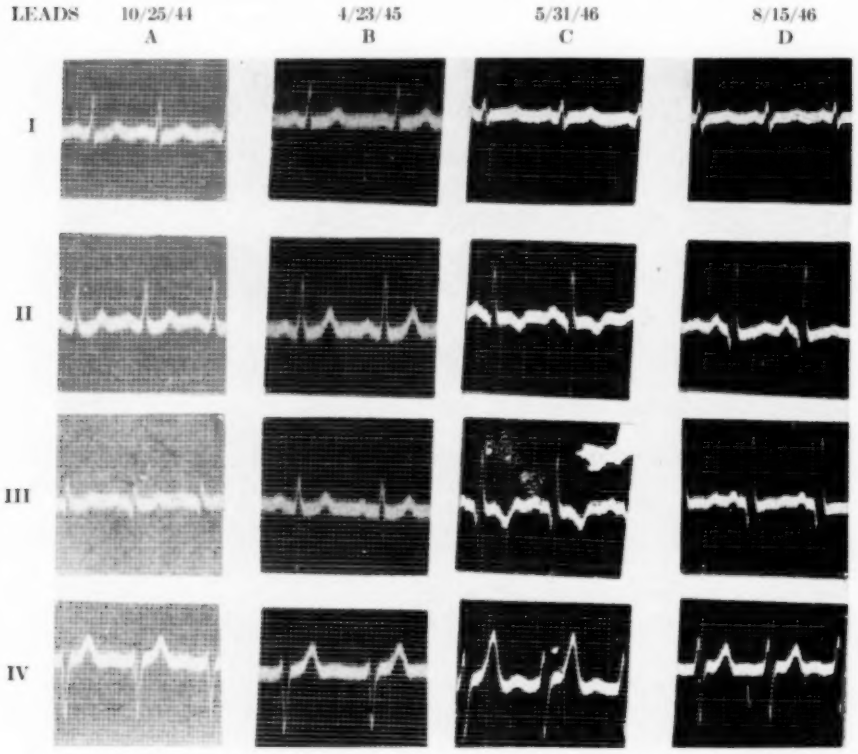

FIG. 6. Case 6 (Age 54). This patient was admitted with bilateral pulmonary tuberculosis with cavitation on the left, and a borderline hypertension with a blood pressure of 150/100. A three-stage left thoracoplasty was done between October 1944, and April 1945. On May 30, 1946, he developed substernal pain of a severe nature with radiation to the left arm. An electrocardiogram taken at this time revealed a tracing compatible with that of a posterior myocardial infarction. (A) Before surgery—normal tracing; (B) following surgery—no change; (C) Q_2T_2, Q_2T_2 pattern (posterior occlusion); (D) Q_2 and Q_2 persistent; T_2 upright; T_2 diphasic.

whether the cause be effusion, adhesions, position of the patient, or surgical measures, and is not considered indicative of heart disease *per se*. This fact has been reported and verified many times (1, 4, 5, 6, 11).

In the 10 cases in the series in which there was shifting of the mediastinum, there were only 2 in which the T-waves in Lead I became of low amplitude, and in

only one was the T-wave in Lead I inverted. Changes in posture from recumbency to sitting did not alter the observed patterns in Leads II, III, and IV in these patients (13). As noted in table 1, however, over half of the group (62.5 per cent) did reveal changes postoperatively in Leads II, III, or IV, either alone or in combination. The T-wave changes observed in Leads I and IV, however,

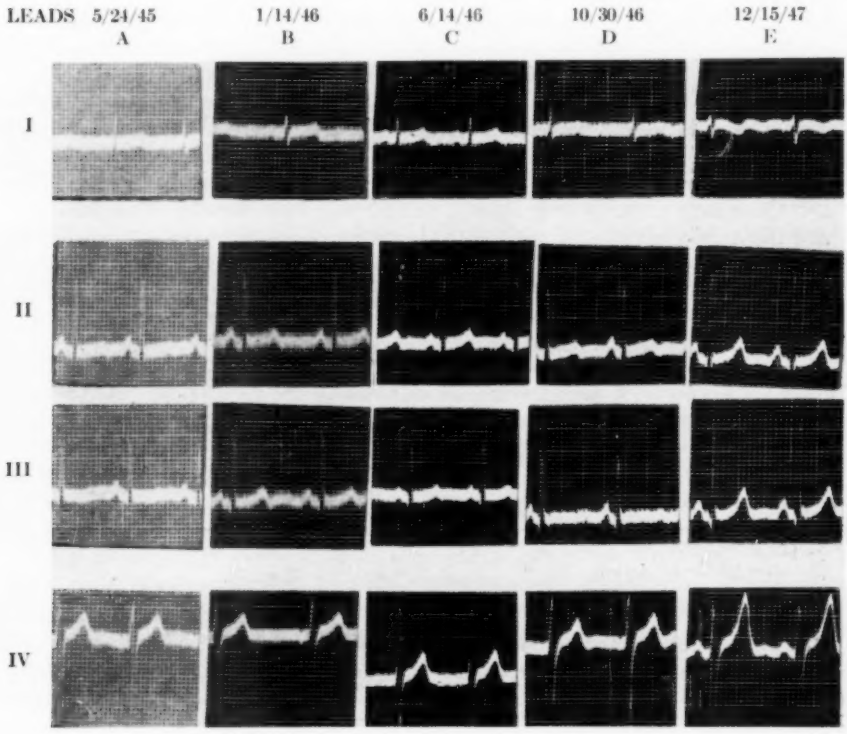

FIG. 7. Case 7 (Age 26). Roentgenogram revealed fibro-exudative disease with cavitation on the left. A two-stage left thoracoplasty was performed between April 13 and May 4, 1945. Electrocardiographic changes were first noted on October 30, 1946. Subsequently this patient had a revision thoracoplasty on November 1, 1946. He had a left lower lobectomy January 1, 1946.

(A), (B), (C), (D) Following 2-stage thoracoplasty—normal tracing; (E) following revision of thoracoplasty—T_1 inverted; T_4 shows high amplitude.

were of paramount concern as they were so striking, tended to persist, and yet did not appear to be due to myocardial damage.

During the course of the study, whenever T_1 inversion was found to have occurred, either alone or in combination with an altered T_4, a careful scrutiny was made for any clinical evidence of heart disease. This included a search for cor-

onary insufficiency, any marked variation in blood pressure, change in heart sounds or rhythm, diminution in cardiac reserve, and especially for evidence of cor pulmonale. These procedures were carried out in an attempt to account for the electrocardiographic changes in Leads I and IV on the basis of demonstrable organic heart disease. It is felt that if heart disease of a nature serious enough to produce the electrocardiographic changes noted were actually present, some clinical evidence thereof would have manifested itself during the early postoperative stages, or certainly within the follow-up period.

Although changes in many instances were pronounced when first noted, they were not considered sufficient to contraindicate subsequent chest surgery. The early experience in this question was obtained by observation of the satisfactory postoperative course followed by several patients for whom surgery was absolutely necessary despite electrocardiographic changes. As already mentioned, these later stages of surgery were well tolerated from a cardiovascular standpoint and there appears to be no justification for assuming the presence of heart dis-

TABLE 2

T-wave changes subsequent to phrenemphraxis in twenty-three patients

T-WAVE CHANGE TYPE OF COLLAPSE	T_1 (I)	T_1 (L)	T_1 (D)	T_2 (I)	T_2 (L)	T_2 (D)	T_3 (I)	T_3 (L)	T_3 (D)	T_4 (I)	T_4 (L)	T_4 (D)	TOTAL NUMBER OF CHANGES
Phrenemphraxis right	1	0	2	1	0	0	0	2	0	1	0	1	8
Phrenemphraxis left..	0	0	2	0	0	0	0	0	1	1	0	1	5

(I) = inversion.
(L) = low or isoelectric.
(D) = diphasicity.

ease merely on the strength of electrocardiographic changes which have not been described in the absence of heart disease prior to this time (2, 7).

DISCUSSION

The reasons for these T-wave changes are not known at present, just as the reasons for inversions of T_2 and T_3 were not known when this phenomenon was first observed in normal individuals. There is no adequate justification for assuming that any toxins accompanying the tuberculous process should suddenly cause severe heart damage with the electrocardiographic changes as noted. Nor were there any postoperative pneumonias or spreads of disease, such as have been reported as possible etiologic factors by Thomson *et al.* (12), which occurred to account for the T-wave changes. The drugs used postoperatively included morphine sulfate; barbiturates, as hypnotics; multiple vitamins; penicillin and streptomycin. It is known that none of these drugs with the possible exception of streptomycin, will produce the changes recorded. Although investigation of the toxic side effects of streptomycin is far from exhausted, a review of the electrocardiograms of over 200 patients who have received the drug has revealed no

similar changes correlated with its administration. Furthermore, only approximately 50 per cent of patients in whom the electrocardiographic changes were found received streptomycin.

The conditions in the present study are far from analogous to those which existed during the early formative years of electrocardiography. Also the type of patient studied is very dissimilar from the "normals" utilized in so many of the earlier studies. The group in the present study consisted predominantly of seriously ill patients who were subjected to major surgery. Such a select group might well be expected to develop some form of heart strain or permanent myocardial damage. Yet this did not occur or, at least, was not evident in a clinically recognizable degree. Thus it seems reasonable to postulate primarily mechanical "extracardiac" etiologic factors as responsible for the electrocardiographic changes observed. Among these may be included obstructive circulatory phenomena, change in position of the heart with and without compression, and postoperative adhesions. In the two cases in the present series which came to autopsy and were available for study, no adhesions were found between the pleura and the pericardium or between the pericardium and the heart. It is recognized that this small number of cases does not constitute a basis for any definite conclusions in this regard.

It has been suggested by Levinson *et al.* (5, 11) that the presence of air in the chest after pneumothorax might well be the cause for the T-wave changes noted in their series, while Simon and Baum (4) postulate that adhesions comprise the significant etiologic factor in producing ventricular predominance. In the present series the only air in the chest of the patients was occasional postoperative subcutaneous emphysema and pneumothorax was not present. It was observed, however, that in those cases in which there was axis deviation (table 3), a definite shift or rotation of the heart and the mediastinum had taken place.

Although some of the electrocardiographic patterns suggest that of a coronary occlusion, none is typical and the argument that a "silent" coronary attack occurred is considered unlikely, primarily on the following basis:

1. There were no signs of coronary insufficiency, even with subsequent major surgery.
2. In general, patients were in the middle of the third decade or early fourth decade and therefore were far less likely to have a coronary occlusion than an individual in an older age group.
3. Of greatest significance, further check with chest leads failed to corroborate the possibility of coronary occlusion.

If the T-wave changes in Leads I and IV were due to an acute coronary insufficiency precipitated during surgery as a result of massive blood loss, they should have been of a transient nature with reversal to normal as soon as there was an elimination of the precipitating factors (10). Multiple transfusions during and after surgery (which was performed under cyclopropane and ether anesthesia, thus permitting optimum oxygenation) coupled with careful postoperative care including the administration of oxygen, certainly served to correct any cardiac anoxia which may have existed during surgery.

Although no careful neuropsychiatric evaluation was made to elicit any deep-

seated emotional conflicts that might have been present, the average patient was not of an abnormally tensive nature, nor possessed of any profound anxiety state which could conceivably contribute to some of the changes noted. If anything, "spes phthisica" was the prevalent attitude.

Only after evaluation of the manifold considerations discussed above did the writer feel justified in accepting the initial impression *i.e.*, that the electrocardiographic changes in Leads I and IV, ordinarily interpreted as signifying organic heart disease, were occurring and persisting in complete absence of myocardial damage as more than a mere hypothesis. At the very least, in the interest of a conservative, open-minded approach, it may be stated that these electrocardiographic changes occurred in the absence of "clinical and other laboratory evidence" of organic heart disease.

It is hoped that these findings will serve to stimulate further investigation along these lines. Recent work at this institution is continuing to afford support to the above formulation. The opportunity of studying such a large group is

TABLE 3

Axis deviation occurring after chest surgery

(Each case with a deviation of axis showed a concurrent shift of the mediastinum)

TYPE OF COLLAPSE	NUMBER OF CASES	RAD	LAD
Thoracoplasty left	10	2	8
Thoracoplasty right	10	5	5
Lobectomy	8	1	0
Pneumonectomy	3	1	1

RAD = right axis deviation.
LAD = left axis deviation.

definitely unique, and the current policy of including an electrocardiographic tracing as part of the routine admission examination record at this hospital is expected to bear fruit over the next several years. Further correlative studies on this series now in progress include amplitude measurements of various phases of the complexes and tabulation of P-wave changes. These findings will be reported subsequently.

SUMMARY

1. A correlation study was attempted between the clinical course and the electrocardiograms made before and after chest surgical procedures in 112 patients.

2. T-wave changes in Leads I and IV, of a type usually considered to indicate severe heart damage, were found to be present in 30 per cent of the entire series of patients. The changes, consisting of either an inversion of T_1 alone or in conjunction with an inverted T_4, occurred in the complete absence of demonstrable heart disease.

3. Arguments are offered to refute the assumption of the presence of coexistent myocardial damage in these patients.

4. A plea is made for further investigation of the changes recorded.

SUMARIO

Significativas Alteraciones Electrocardiográficas Consecutivas a la Cirugia Torácica

1. Este estudio tuvo por mira correlacionar la evolución clínica y los electrocardiogramas obtenidos antes y después de operaciones torácicas en 112 enfermos.

2. En 30 por ciento de toda la serie de enfermos descubriéronse modificaciones en las Derivaciones I y IV de la onda en T, en forma de inversión de la T_1, ya sola o unida a inversión de la T_4, en ausencia completa de toda cardiopatía observable.

3. Ofrécense argumentos para confutar la suposición de la coexistencia de alteraciones miocardíacas en estos enfermos.

4. Abógase por que continúen las investigaciones de las alteraciones descritas.

Acknowledgments

The writers are indebted to J. D. Murphy, M. D., Chief of the Surgical Service, and his staff for their indispensable services in making this study possible, and to Mrs. Mildred Knowlton, Medical Librarian, for valuable assistance in translation of foreign articles and preparation of the final manuscript.

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STREPTOMYCIN-TUBERCULOSIS RESEARCH PROJECT OF THE AMERICAN TRUDEAU SOCIETY[1,2,3,4]

A Summary Report

H. McLEOD RIGGINS[5] AND H. CORWIN HINSHAW[5]

INTRODUCTION

In the fall of 1946, after it had been demonstrated (1, 2, 3, 4, 8, 9) that streptomycin had definite therapeutic potentialities in the treatment of clinical tuberculosis, the manufacturers of streptomycin donated a large quantity to the American Trudeau Society for expansion of clinical research. The primary purpose of the investigation was to explore the integration of streptomycin therapy with other therapeutic procedures in the treatment of *pulmonary* types of the disease. Although much had already been learned concerning the limitations and therapeutic potentialities of streptomycin in the treatment of tuberculosis, nevertheless there still remained many questions only partially answered. Some of the major problems were: (*1*) determination of the types of pulmonary tuberculosis most likely to benefit from the use of streptomycin; (*2*) the most satisfactory methods and frequency of treatment; (*3*) the optimum daily dosage; (*4*) the optimum duration of treatment; (*5*) the most timely integration of streptomycin with other therapeutic procedures, especially collapse therapy and lung resection; (*6*) the determination of the incidence and clinical significance of the emergence of strains of tubercle bacilli less sensitive to the drug and how this phenomenon might be delayed, minimized or prevented; (*7*) the incidence, severity, clinical significance and, if possible, the prevention of serious toxic manifestations, particularly deafness and vestibular dysfunction; and (*8*) relapse of the disease.

The Executive Committee of the American Trudeau Society designated a group of clinical and laboratory investigators, who later functioned as Clinical and Laboratory Chemotherapy Subcommittees of the Committee on Medical Research and Therapy, to collaborate in carrying out studies on the above and other problems. Members of the Clinical Chemotherapy Subcommittee were: Dr. J. Burns Amberson, New York, New

[1] The full report, including chapters by those participating in the project and by others on certain fundamental aspects of the problem, is now being compiled and edited for publication in book form by the National Tuberculosis Association.

[2] For previous report refer to H. McLeod Riggins and H. Corwin Hinshaw, The Streptomycin-Tuberculosis Research Project, American Trudeau Society, Am. Rev. Tuberc., 1947, *56*, 168.

[3] This study was aided by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and from the Medical Research Committee of the National Tuberculosis Association.

[4] The streptomycin was generously donated by Abbott Laboratories, Eli Lilly and Co., Merck and Co., Inc., Chas. Pfizer and Co., E. R. Squibb & Sons, and The Upjohn Company.

[5] Present (1947–1948) and previous (1946–1947) Chairmen of the Subcommittee on Chemotherapy, Committee on Medical Research and Therapy, American Trudeau Society.

York; Dr. Emil Bogen, Olive View, California; Dr. H. Corwin Hinshaw, Rochester, Minnesota (Chairman 1946–1947); Dr. Kirby S. Howlett, Jr., Shelton, Connecticut; Dr. Walsh McDermott, New York, New York; Dr. Edward N. Packard, Trudeau, New York; Dr. H. McLeod Riggins, New York, New York (Chairman 1947–1948); and Dr. H. Stuart Willis, McCain, North Carolina.

Members of the Laboratory Chemotherapy Subcommittee were: Dr. Emil Bogen, Olive View, California; Dr. H. Corwin Hinshaw, Rochester, Minnesota; Mr. William Steenken, Jr., Trudeau, New York; Dr. H. Stuart Willis, McCain, North Carolina; Dr. C. Eugene Woodruff, Northville, Michigan; and Dr. Guy P. Youmans, Chicago, Illinois (Chairman).

In order to coordinate the laboratory studies in connection with the project and to ensure more adequate facilities and uniform procedures, three district laboratories were designated at: Olive View Sanatorium, Olive View, California, Dr. Emil Bogen, Director; Trudeau Sanatorium, Trudeau, New York, Mr. William Steenken, Jr., Director; and Northwestern University Medical School, Chicago, Illinois, Dr. Guy P. Youmans, Director.

The present communication is in no way to be construed as a comprehensive report covering the studies of the individual investigators, but rather as a report in summary form including discussions and tentative opinions with regard to some of the more important aspects of the study. A comprehensive report, including chapters by the individual investigators and more extensive tabular and graphic data, will be published in book form at an early date by the National Tuberculosis Association.

OBSERVATIONS

Clinical Material

A total of 566 patients were studied between September, 1946 and January, 1948. Information on these patients was obtained from the eight investigators through the medium of questionnaires. A comprehensive statistical analysis was carried out on the pertinent findings in 332 patients with pulmonary tuberculosis who had completed streptomycin therapy and had been observed for at least 90 days after the beginning of treatment. Reports on the remaining 234 patients are not included in the present analysis because they were either treated for less than 30 days, had been observed for less than 90 days after the beginning of treatment (at the time the questionnaires were returned), had received less than 0.5 Gm. of streptomycin daily, or were patients with meningeal or miliary tuberculosis or had some type of extrapulmonary tuberculosis only.

Of the 332 patients with pulmonary tuberculosis reported on at this time, 17 had minimal, 118 moderately advanced and 197 far advanced disease. Eighty per cent of these patients were stated by the investigators to have predominantly acute or subacute disease, and the remainder predominantly chronic disease at the beginning of streptomycin treatment (figure 1).

One hundred seventy-seven of these 332 patients with pulmonary tuberculosis had a total of 204 known tuberculous complications. The principal complications were: (*1*) tracheobronchial and laryngeal tuberculosis, 125; (*2*) lymph

node and sinus tract tuberculosis, 18; (*3*) intestinal tract tuberculosis, 16; (*4*) bone and joint tuberculosis, 13; (*5*) genito-urinary tract tuberculosis, 10; and (*6*) miscellaneous complications, 22.

Grouping of the 332 patients according to race, sex, and age showed the following: white, 262; Negro, 36; other, 34; female, 200; male, 132. Sixty-six per cent of the patients were between 20 and 39 years of age.

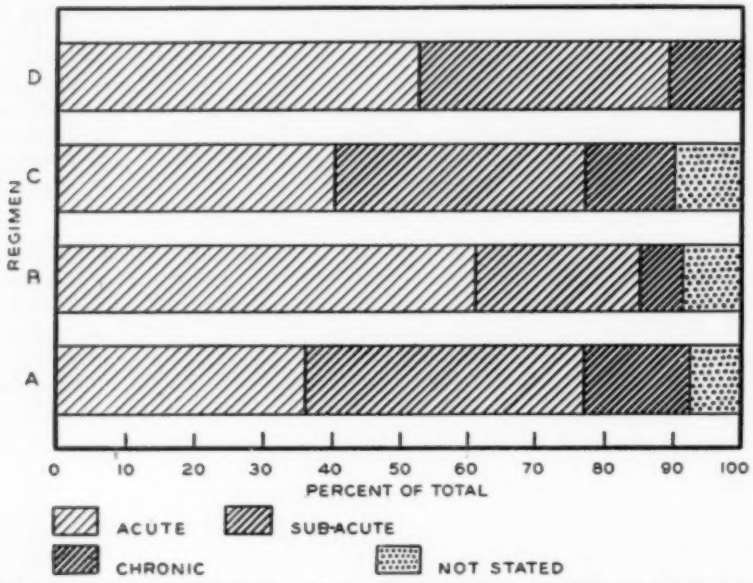

FIG. 1. Clinical types of disease, at beginning of streptomycin therapy, in 332 patients with pulmonary tuberculosis (according to regimen).

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

Relationship of Streptomycin Efficacy to the Type and Extent of Pulmonary Pathology

Before considering the comparative therapeutic advantages and limitations of different streptomycin regimens, consideration should be given to the more important basic factors which significantly influence or largely determine the therapeutic potentialities of any antibacterial drug in tuberculosis. These factors are the character and extent of the predominant tuberculous disease, the resulting physiologic abnormalities, the immediate and remote potentialities of the disease,

the native and acquired resistance of the patient, and other factors which may significantly limit the successful application of a comprehensive and properly integrated therapeutic program. The mechanism of action of streptomycin in inhibiting the disease process affords additional insight into the potentialities and limitations of streptomycin.

The beneficial effect of streptomycin in tuberculosis is dependent upon its interference with the growth and multiplication of streptomycin-sensitive strains of *M. tuberculosis* (5). Streptomycin apparently does not directly enhance tissue repair but it probably does favorably influence the tempo and quality of repair by inhibiting the growth and multiplication of tubercle bacilli. Assuming this to be true, it would be expected that the response of the tuberculous lesion to streptomycin would be fundamentally related to the characteristics of the pathology and the patient's ability to cope with his disease. It has been shown, experimentally and clinically, that streptomycin is more effective against relatively early acute and subacute lesions, in which the extent and degree of caseation is limited, than it is against confluent and rapidly caseating and liquefying tuberculous pneumonias, or against extensive chronic fibrocaseous and cavernous disease. Under certain circumstances, therefore, streptomycin appears to bring into favorable balance resistance and repair over susceptibility and destruction. In view of the mechanism by which streptomycin appears to affect certain tuberculous lesions beneficially, it cannot be expected that the drug will exert a direct favorable influence on such irreversible pathological lesions as extensive caseation and fibrosis. It may logically be expected, however, that streptomycin can favorably influence the behavior of some pulmonary cavities, especially those which tend to increase in size as the result of progressive inflammation, caseation, and liquefaction, or certain of those whose size and behavior are influenced by acute and ulcerative tuberculous bronchitis. If the behavior of the pulmonary cavity is related to an active tuberculous bronchitis, healing of the bronchial disease and the re-establishment of normal bronchial function, as a result of streptomycin therapy, may significantly aid closure and healing of the cavity.

These fundamental factors, often minimized or overlooked entirely, play a more decisive role in the degree of therapeutic effectiveness of streptomycin and the ultimate outcome of the issue than do the often overemphasized differences in total daily dosage or duration of treatment, as important as these latter factors are. In this connection, it should be stressed that in advanced progressive chronic tuberculosis the present clinical and roentgenologic methods for determining the different types of pathological processes in their true proportions, and classifying them, are relatively crude and inaccurate at best. The effect of streptomycin has frequently emphasized the limitations of these methods. Frequently, disease presumed to be predominantly caseous and at other times predominantly productive undergoes marked resolution and clearing and, perhaps equally often, disease presumed to be largely inflammatory in nature proves to be caseous. The practical difficulties, therefore, of choosing essentially comparable types of disease, particularly from the point of view of the effect of a chemotherapeutic agent, in equal proportions in different regimens become clear.

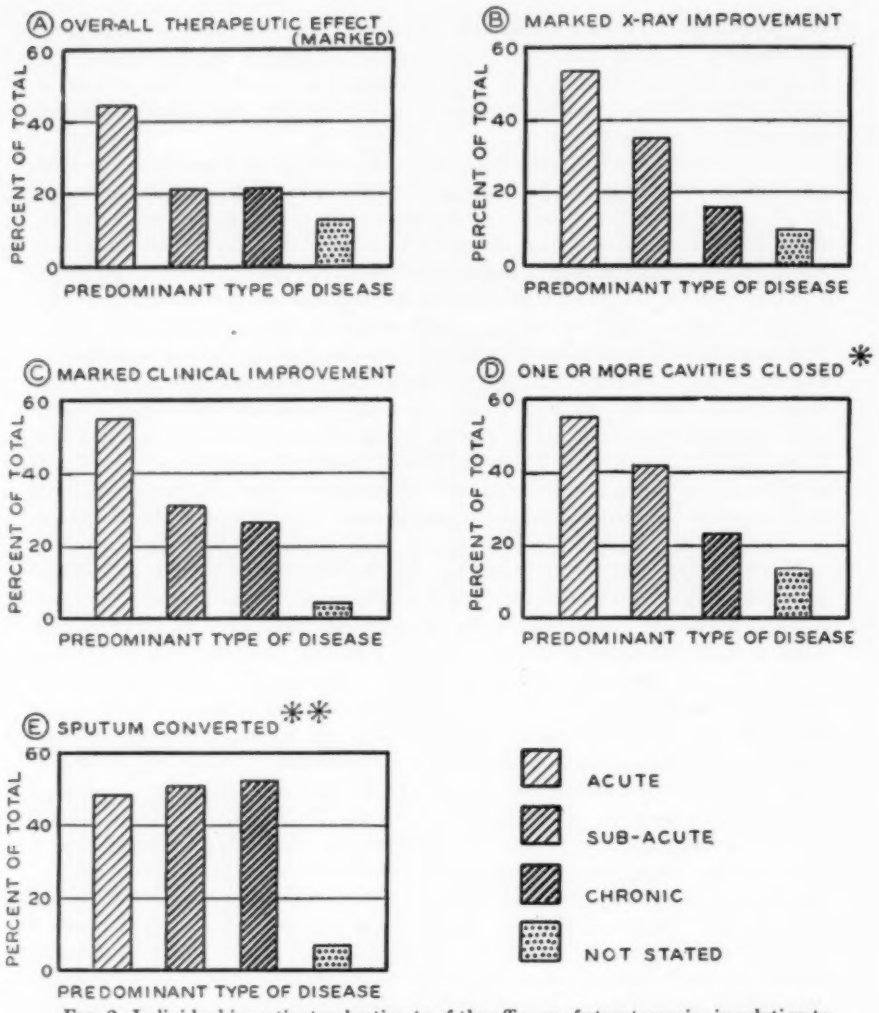

FIG. 2. Individual investigators' estimate of the efficacy of streptomycin, in relation to the predominant type of disease, in 332 patients with pulmonary tuberculosis, observed from six to ten months after the beginning of treatment.

* Percentages based upon 218 patients with demonstrable cavities at the beginning of streptomycin treatment.

** Percentages based upon 284 patients with sputum or gastric lavage contents positive for tubercle bacilli.

It is equally obvious that erroneous conclusions may be drawn if the differences in the results obtained are solely attributed to the differences in regimens used. This is especially true if small numbers of patients are involved.

An attempt was made to evaluate the therapeutic effect of streptomycin on the different clinical types of disease in 332 patients with pulmonary tuberculosis classified by the investigators as predominantly acute or subacute and predominantly chronic. The results are shown in figure 2. The effectiveness of the antibiotic on the three types of disease was measured in terms of: (*1*) clinical improvement, (*2*) roentgenographic improvement, (*3*) closure of cavities, (*4*) "conversion of sputum," and (*5*) over-all marked improvement. In general, the therapeutic effectiveness of streptomycin was strikingly greater in patients with recent and presumably acute exudative disease. The drug also appeared to be more effective against subacute than against chronic disease. The degree of its effectiveness against subacute disease, however, was not as consistent or striking as against the more recent and acute exudative disease. The least beneficial effects were noted in patients with extensive predominantly chronic disease.

One of the significant limitations of streptomycin, with regard to all three types of disease, and irrespective of the regimen used, was emphasized by the similarity of the sputum findings. There was no significant difference found in "sputum conversion" in the different types of disease.

As the degree of effectiveness of streptomycin is fundamentally related to the type and extent of pathology, and as it appears to be least effective against extensive chronic disease, it should not be anticipated that streptomycin and bedrest alone would bring about arrest of the disease in the great majority of patients with advanced chronic cavitary tuberculosis.

Streptomycin Regimens

None of the investigators was restricted to the exploration of a single regimen and as a rule each investigator used several different regimens. A few investigators explored many regimens. Because of the multiplicity of regimens employed it was impossible to evaluate all of them individually. Therefore, for statistical comparison of four basic regimens, the 332 patients with pulmonary tuberculosis who were observed for at least ninety days after the beginning of treatment were arbitrarily divided into four groups.

Regimen A. 124 patients	Patients treated for 30 to 89 days, with a daily dosage of streptomycin varying from 0.5 Gm. to 1.4 Gm.
Regimen B. 46 patients	Patients treated for 30 to 89 days, with a daily dosage of streptomycin varying from 1.5 Gm. to 3.0 Gm.
Regimen C. 89 patients	Patients treated for 90 days or over, with a daily dosage of streptomycin varying from 0.5 Gm. to 1.4 Gm.
Regimen D. 73 patients	Patients treated for 90 days or over, with a daily dosage of streptomycin varying from 1.5 Gm. to 3.0 Gm.

The most important facts brought out in table 1 are: (*1*) Of the 213 patients receiving daily dosages varying from 0.5 Gm. to 1.4 Gm., 148 (70 per cent) received 1.0 Gm. daily. (*2*) Of the 119 patients receiving daily dosages varying from 1.5 Gm. to 3.0 Gm., only 10 (8 per cent) received 3.0 Gm. daily. In the evaluation of results according to regimens it is important to bear in mind the fact that a great majority of the patients in regimens A and C received a daily dosage of 1.0 Gm. and in regimens B and D only a few patients received 3.0 Gm. daily.

TABLE 1

Breakdown of total patients in each regimen according to average daily dosage

AVERAGE DAILY DOSAGES	REGIMEN A NUMBER OF PATIENTS	REGIMEN B NUMBER OF PATIENTS	REGIMEN C NUMBER OF PATIENTS	REGIMEN D NUMBER OF PATIENTS
0.5 Gm.	12	—	8	—
0.6 to 0.9 Gm.	12	—	10	—
1.0 Gm.	94	—	54	—
1.1 to 1.4 Gm.	6	—	17	—
1.5 to 1.9 Gm.	—	13	—	34
2.0 to 2.9 Gm.	—	24	—	38
3.0 Gm.	—	9	—	1
Total	124	46	89	73

To evaluate different regimens providing different durations of treatment with accuracy, it is essential to have the results analyzed at the end of *comparable* periods of time after the *beginning* of streptomycin therapy. In this study, results obtained in the treatment of patients with regimen A were analyzed an average of 182 days after the beginning of treatment; with regimen B, an average of 243 days after the beginning of treatment; with regimen C, an average of 264 days after the beginning of treatment; and with regimen D, an average of 294 days after the beginning of treatment.

Any suggestions, trends, or tentative opinions gained from such an analysis as this must of necessity be considered in the light of the inherent and obvious deficiencies of this and similar cooperative studies involving a number of investigators. This project does have certain qualities to recommend it, however, a few of which might be noted. The investigation represents the combined thinking and efforts of a coordinated national group of clinical and laboratory investigators associated with representative medical schools and tuberculosis teaching centers. The clinical material consists of a wide sampling of pulmonary tuberculosis from the point of view of the different types of disease, age, race, and sex of the patients treated in widely separated sections of the country. All information and opinions concerning individual patients, including evaluation of results, are entirely those of the individual investigators. The compiling and tabulating of the statistical material were carried out by Dr. Carroll E. Palmer and Mrs. Shirley H. Ferebee and their staff of the U. S. Public Health Service. None of this group had an official connection with the streptomycin study of the American Trudeau Society. Finally, the number of patients observed was considerably larger than could have been studied by a single investigator at the time and is considered sufficiently large to be of statistical significance.

Evaluation of Streptomycin Regimens

Evaluation of the results obtained on the four regimens is based upon the reports of the individual investigators concerning clinical and roentgenographic

findings, closure of cavities, "conversion of sputum," estimates of the over-all therapeutic effect of the drug, toxic manifestations of streptomycin, emergence of strains of tubercle bacilli less sensitive to streptomycin, and relapse of the disease. As the results could have been partially affected by such other therapeutic procedures as bed-rest and collapse therapy, the value of streptomycin was estimated in each instance by the physician treating the patient.

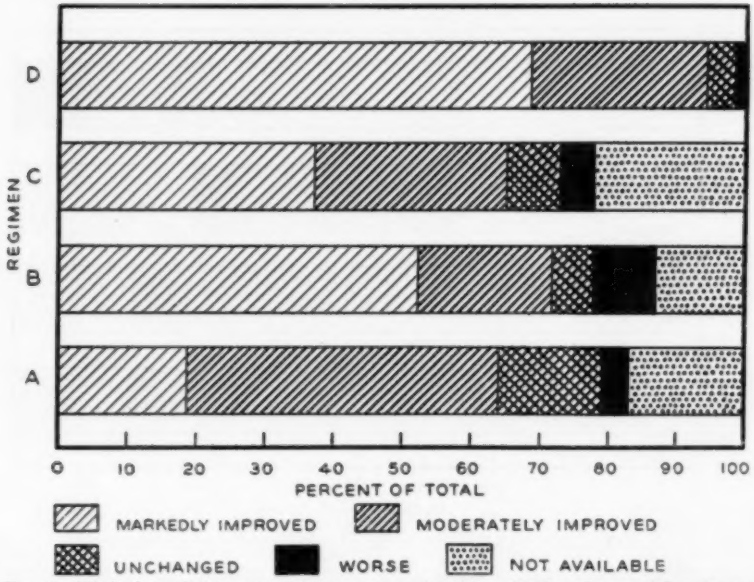

FIG. 3. Clinical condition, at most recent examination, of 332 patients with pulmonary tuberculosis observed from six to ten months after start of streptomycin, in comparison with beginning of treatment (according to regimen).

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

Daily Dosage and Duration of Treatment

Despite the fundamental factors which influence the effectiveness of streptomycin, a comparison of the results obtained on the four different regimens appears to indicate some relationship between the size of the daily dosage and the therapeutic results attained. Moreover, a similar though less definite relationship appears to exist between the duration of treatment and the therapeutic results. It is to be re-emphasized, however, that the relationship which appears to exist

between therapeutic results and daily dosage may be more *apparent* than *real*, because of the difference in the percentage distribution of the clinical types of cases treated in the four regimens (figure 1) and for the other reasons previously mentioned.

Clinical improvement: As may be seen in figure 3, clinical improvement occurred in 86 per cent of the patients receiving the larger daily dosages (1.5 Gm. to 3.0 Gm., regimens B and D) in contrast to 65 per cent of the patients who

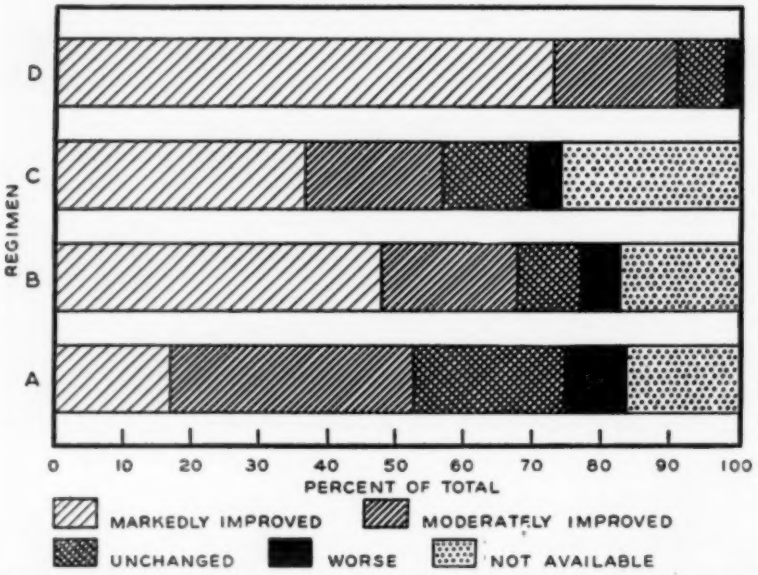

FIG. 4. Condition, according to most recent roentgenological findings, of 332 patients with pulmonary tuberculosis observed from six to ten months after start of streptomycin, in comparison with beginning of treatment (according to regimen).

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

received the smaller daily dosages (0.5 Gm. to 1.4 Gm., regimens A and C). Clinical improvement seems related *much less* to duration of treatment, in so far as a comparison of the two periods of treatment used (30 to 89 days, and 90 days or over) is concerned. In the patients receiving treatment for the longer periods of time (90 days or over, regimens C and D) 78 per cent showed clinical improvement, while in the patients receiving treatment for the shorter periods of time (30 to 89 days, regimens A and B) 66 per cent showed clinical improvement.

Roentgenographic improvement: The frequency and degree of the over-all roentgenographic improvement seem to be related to the total daily dosage (figure 4). This is shown by comparing the results obtained on regimens A and B, C and B, and C and D. In each instance it will be seen that the best results were obtained in patients receiving the larger daily dosages. Comparatively better results were obtained on regimen B than on regimen C, even though pa-

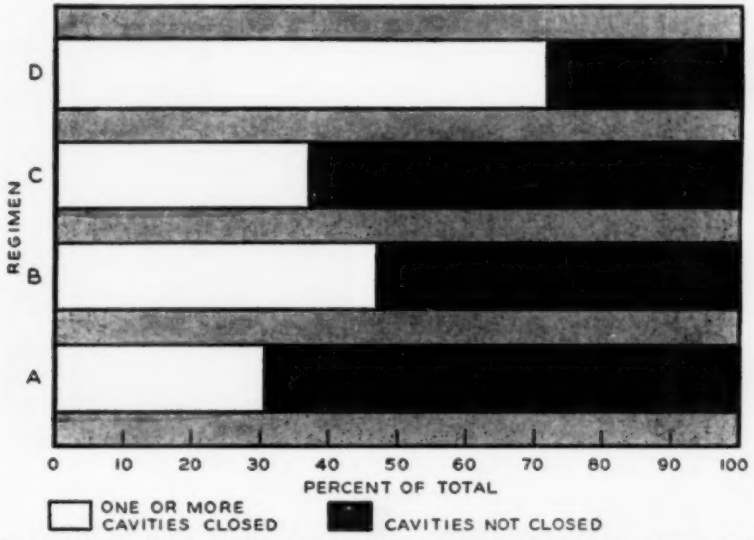

FIG. 5. Closure of one or more cavities, during and after streptomycin therapy, in 218 patients with pulmonary tuberculosis observed from six to ten months after the beginning of treatment (according to regimen).

(Only 218 of the 332 patients had demonstrable cavities at the beginning of streptomycin therapy.)

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

tients on the latter regimen were treated for a longer period of time. Roentgenographic improvement was noted in 82 per cent of the patients receiving the larger daily dosages (regimens B and D) and in 54 per cent of the patients receiving the smaller daily dosages (regimens A and C). The over-all roentgenographic improvement seems also to be related, but to a *much less* extent, to the duration of treatment. This is shown by comparing the combined results obtained on regimens A and B with the combined results on regimens C and D. Roentgeno-

graphic improvement was noted in 72 per cent of the patients receiving treatment for the longer periods of time (90 days or over, regimens C and D), and in 56 per cent of the patients who received treatment for the shorter periods of time (30 to 89 days, regimens A and B).

Closure of cavities: In general, the study revealed that the same relationship appears to exist between the closure of cavities and the total daily dosage and

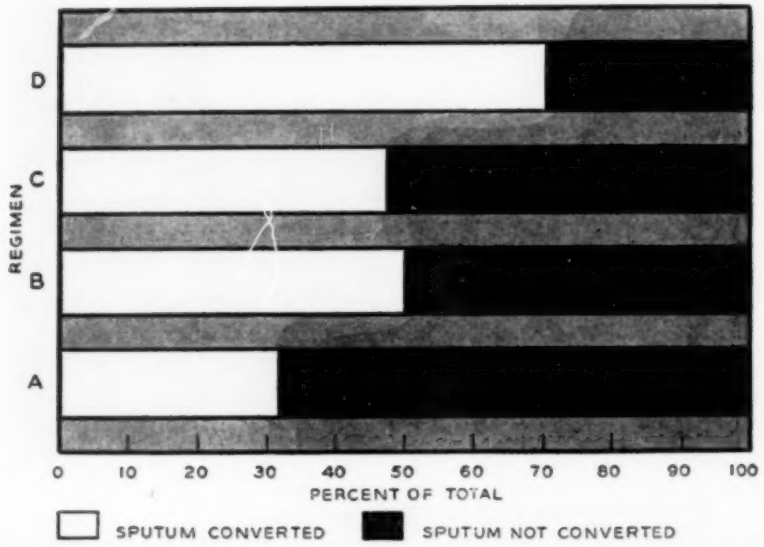

FIG. 6. Conversion of sputum from positive to negative for tubercle bacilli, during or after streptomycin therapy, in 284 patients with pulmonary tuberculosis observed from six to ten months after the beginning of treatment (according to regimen).

(The remaining 48 patients had recently had sputum or gastric lavage contents positive for tubercle bacilli.)

Regimen	*Duration o Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

duration of treatment as was found in analysis of the over-all roentgenographic and clinical improvement (figure 5). That is to say, the closure of one or more cavities occurred in 62 per cent of those patients who received the larger daily dosages of streptomycin in contrast to 34 per cent in the patients receiving the smaller daily dosages. Closure of one or more cavities occurred in 51 per cent of patients receiving treatment for the longer periods of time (90 days or over, regimens C and D), while it occurred in 36 per cent of the patients receiving

treatment for the shorter periods of time (30 to 89 days, regimens A and B). Follow-up of some of these patients, however, has shown that cavities which disappeared on the chest roentgenogram or were apparently closed have reopened. Such findings provide support for the opinion that streptomycin is usually not a definitive form of therapy, especially for the more destructive and chronic types of tuberculosis. If durable results are to be obtained, it is always

FIG. 7. Individual investigators' estimate of the over-all efficacy of streptomycin in 332 patients with pulmonary tuberculosis, observed from six to ten months after the beginning of treatment (according to regimen).

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

essential to supplement streptomycin with bed-rest and frequently with some form of collapse therapy or occasionally lung resection.

"Conversion of sputum": The disappearance of tubercle bacilli from the sputum occurred more frequently in the patients receiving the larger daily dosages of streptomycin (63 per cent) than in the patients receiving the smaller daily dosages (38 per cent). Reversal of infectiousness also appears to be related to the duration of treatment. In the patients receiving treatment for the longer periods

of time (90 days or over) "sputum conversion" occurred in 58 per cent, while in the patients treated for the shorter periods of time (30 to 89 days) "conversion" occurred in 36 per cent.

The unusually high percentage of "sputum conversions" in all regimens may be partially explained by the fact that "conversion" of sputum in this series of patients was based upon the results of the last sputum examination and in many instances the type of examination was by concentration method. The high percentages of "sputum conversion" would probably be much lower if they were based upon repeated examination by culture or animal inoculation.

The follow-up of certain patients whose sputum was "converted" shows that not infrequently the sputum again becomes positive for tubercle bacilli. It is believed that the increment of reopened cavities and the return of infectiousness may increase significantly with the passage of time following completion of streptomycin therapy, particularly if other therapeutic procedures are not properly applied at the opportune time. It should be borne in mind that the results were evaluated from 182 to 294 days after the beginning of streptomycin treatment.

The consistency of the relationship between the total daily dosage and therapeutic results and, to a less degree, between the duration of treatment and therapeutic results is still further suggested by the reports of the individual investigators of the estimated over-all therapeutic effects of streptomycin obtained on the four regimens (figure 7).

Sensitivity of Tubercle Bacilli to Streptomycin before, during and after Completion of Therapy

It has been well established by many workers that human strains of *M. tuberculosis* which have not been previously exposed to streptomycin are sensitive *in vitro* to low concentrations of the antibiotic. Studies have also shown that a high percentage of strains of tubercle bacilli become less sensitive to streptomycin in proportion to the *duration* of exposure to the drug. It has occasionally been noted that organisms still sensitive to streptomycin at the completion of treatment may become less sensitive at some later date and that the degree of "drug fastness" present at the end of treatment may actually increase following cessation of treatment. It is important, therefore, to determine the sensitivity of the organisms routinely before retreatment is undertaken.

The possibility of the existence of a relationship between the daily dosage of streptomycin and the rapidity and frequency of the emergence of significantly resistant strains during treatment (10 or more γ of streptomycin per ml.) has potential implications concerning optimum daily dosage. The few data available regarding this possible relationship will be presented. It is also possible that the simultaneous use of certain tuberculostatic agents with streptomycin may perhaps delay or decrease the emergence of streptomycin-resistant organisms. Should further studies along this line prove encouraging, large scale trials of combined chemotherapy would seem definitely warranted for the purpose of clarifying this question. A few such studies are already under way.

A limited amount of information concerning the emergence of less sensitive

strains of tubercle bacilli during streptomycin therapy is available from this study. As there is increasing evidence that *in vitro* resistance is indicative also of *in vivo* resistance and that microorganism resistance of 10 or more γ of streptomycin per ml. is of some clinical significance, data will be presented only on those patients whose tubercle bacilli became resistant to 10 or more γ of streptomycin. Reliable data concerning microorganism sensitivity during streptomycin treatment are available on 159 of the 332 patients in the series. Data on the remaining patients are incomplete for various reasons but largely because bacilli were not available for testing at the completion of treatment.

Of the 159 patients, 56 (35.2 per cent) are known to have discharged tubercle bacilli resistant to 10 or more γ of streptomycin per ml. at various periods of time after the beginning of treatment. Of 17 patients whose bacilli were studied for streptomycin sensitivity one to 30 days after the start of treatment, 2 (11.7 per cent) had organisms resistant to 10 or more γ per ml.; of 40 patients whose organisms were studied 31 to 60 days from the start of treatment, 12 (30 per cent) had organisms resistant to 10 or more γ per ml.; of 27 patients whose bacilli were studied 61 to 90 days after the start of treatment, 12 (44.4 per cent) had organisms resistant to 10 or more γ per ml.; of 23 patients whose bacilli were studied 91 to 120 days after the start of treatment, 12 (52.2 per cent) had organisms resistant to 10 or more γ per ml.; and of 52 patients whose bacilli were studied 121 days or later after the start of treatment, 18 (34.6 per cent) had organisms resistant to 10 or more γ per ml. In the remaining 103 patients (64.9 per cent) studied periodically during part of the treatment period, the sputum either became negative or the organisms remained sensitive to less than 10 γ of streptomycin per ml. It is believed that, if it were possible to determine the streptomycin sensitivity of tubercle bacilli in all patients at the end of prolonged treatment, there would be a much greater incidence of significantly resistant strains than indicated above.

These data are too few to permit a precise analysis of the relation of the length of treatment to either the incidence or the time of appearance of drug-resistant strains of tubercle bacilli. Nevertheless, examination of the data does reveal that the emergence of what appear to be significantly resistant strains (resistant to 10 or more γ streptomycin per ml.) did occur in a fairly high percentage (35.2 per cent) of the patients.

Possibly of equal importance is the fact that there appears to be some relationship between the total daily dosage and the rapidity of emergence of organisms resistant to at least 10 γ of streptomycin per ml. Although the number of patients in this group is too small to have statistical significance, nevertheless it is of interest to note that the emergence of organisms resistant to 10 γ and over per ml. was more rapid in patients receiving the *larger* daily dosages of streptomycin (1.5 Gm. to 3.0 Gm., regimens B and D) than in patients receiving the smaller daily dosages (0.5 Gm. to 1.4 Gm., regimens A and C) (figure 8). It is probably of equal importance, however, to note that a *greater* percentage of patients who received the *smaller* daily dosages showed emergence of strains resistant to 10 or more γ per ml. Of the 85 patients receiving the *smaller* daily

dosages, 35 (41.2 per cent) had organisms resistant to 10 or more γ per ml. at different periods of time after the start of treatment, and of the 74 patients receiving the larger daily dosages, only 21 (28.4 per cent) had organisms resistant to 10 or more γ per ml. after beginning of treatment.

These data would seem to pose another question with regard to optimum daily dosage. Considered only in the light of the rapidity of emergence of organisms significantly resistant to streptomycin, it might be assumed, if these findings

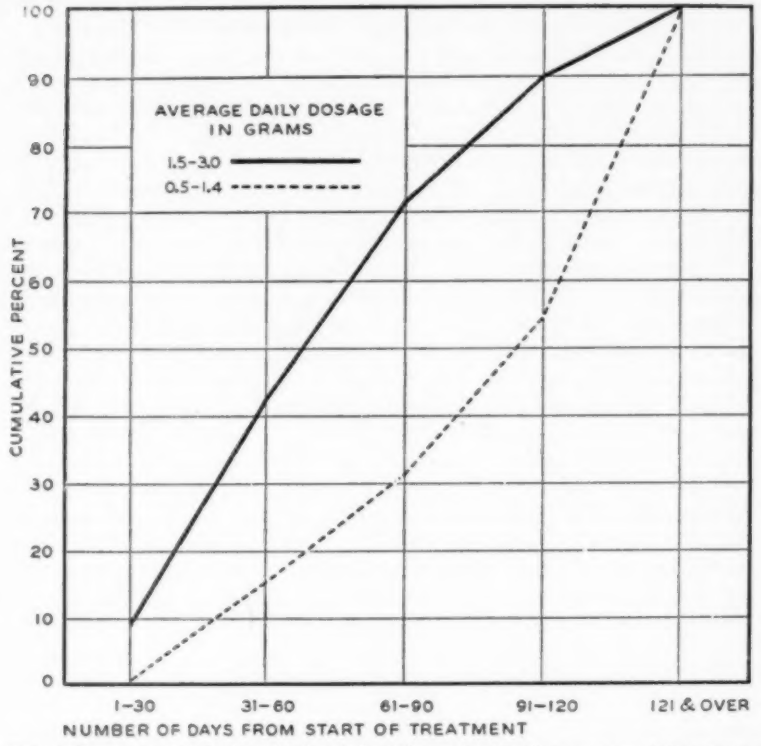

FIG. 8. Emergence of strains of tubercle bacilli resistant to 10 or more γ of streptomycin per ml., in relation to duration of treatment and average daily dosage.

are confirmed, that an advantage of smaller daily dosages is that they presumably cause the emergence of significantly resistant organisms less rapidly than do larger daily dosages. For the group of 56 patients whose microorganisms were resistant to 10 or more γ of streptomycin per ml., the monthly percentages[6] and the cumulative percentage of emergence of this degree of drug-resistance are shown in figure 8.

[6] Sensitivity tests were done periodically from start of treatment.

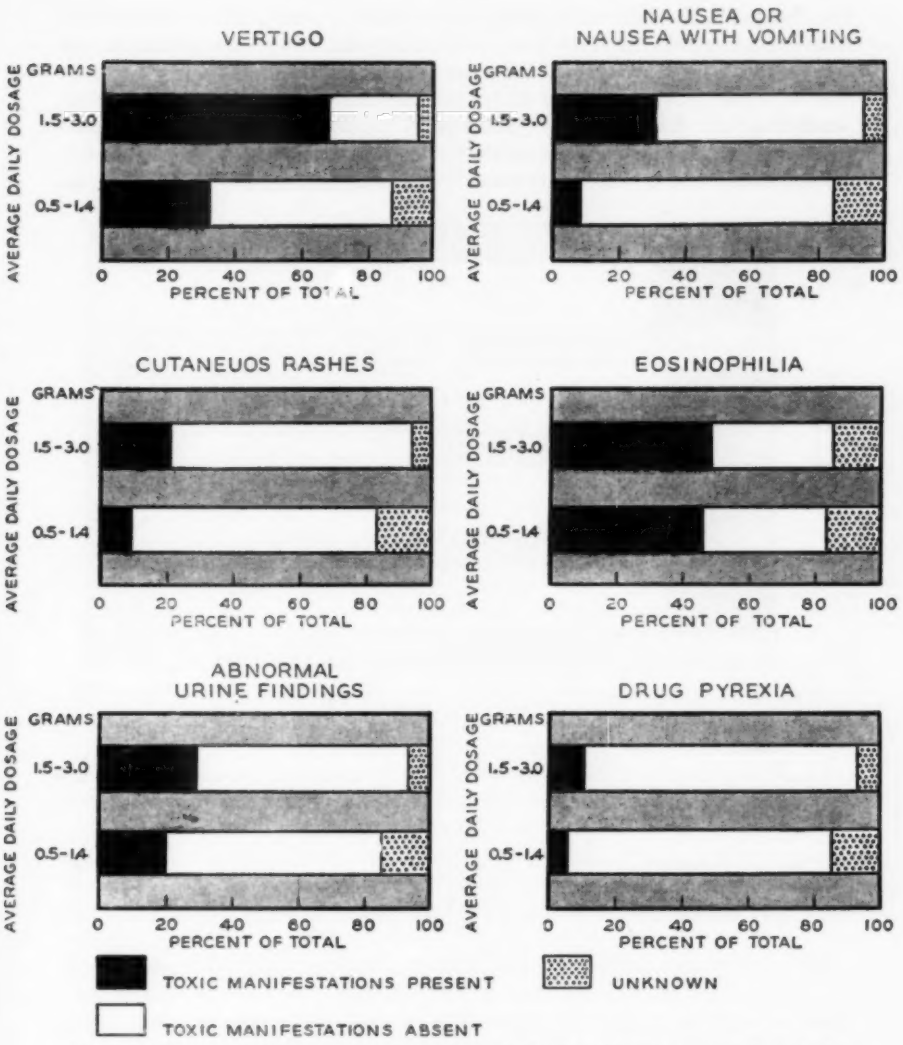

FIG. 9. Toxic manifestations encountered in 332 patients with pulmonary tuberculosis, according to average daily dosages of streptomycin.

Toxic manifestations: The frequency and severity of toxic manifestations were consistently *greater* in the patients receiving the *larger* daily dosages of streptomycin (1.5 Gm. to 3.0 Gm., regimens B and D) than in the patients receiving the smaller daily dosages (0.5 Gm. to 1.4 Gm., regimens A and C) (figure

9). Named in the order of their importance and frequency, the toxic manifestations encountered in this series were: vestibular dysfunction; eosinophilia; nausea and vomiting; evidence of renal irritation or damage which was usually transient (severe renal damage due primarily to streptomycin was not encountered); dermatitis; and drug pyrexia. In the great majority of instances toxic manifestations were reversible, labyrinthine dysfunction usually being either reversible or compensated for in almost all cases. Permanent deafness due to streptomycin

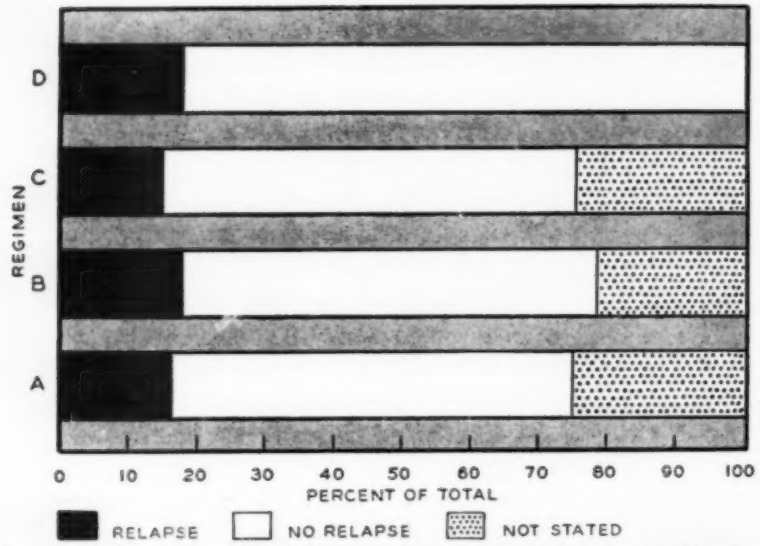

FIG. 10. Relapse of the disease, during or after streptomycin therapy, in 332 patients with pulmonary tuberculosis observed from six to ten months after the beginning of treatment (according to regimen).

Regimen	*Duration of Treatment* (days)	*Daily Dosage Streptomycin* (grams)
A	30 to 89	0.5 to 1.4
B	30 to 89	1.5 to 3.0
C	90 or over	0.5 to 1.4
D	90 or over	1.5 to 3.0

was not reported by the investigators in this series of pulmonary tuberculosis patients.

In the last analysis, determination of the optimum total daily dosage is based upon the possible degree of therapeutic effectiveness, on the one hand, and the possible severity of the toxic effects, on the other. Within certain limits, the selection of the optimum daily dosage is related to the threat of the disease versus the potential untoward toxic effects. For example, it might be pointed out that it is better to use a relatively large dosage of streptomycin and cure a

tuberculous meningitis, even though deafness supervenes, than to use, in attempting to avoid toxic effects, ineffective dosage and lose the patient. The same reasoning does not always apply, however, with regard to the use of larger daily dosage (20 to 40 mg. per kilogram of body weight) for many patients with pulmonary tuberculosis. In these patients the threat of the disease to life is usually not as definite and immediate, nor is streptomycin therapy definitive but must usually be accompanied by other therapeutic procedures if durable results are to be achieved. It is strongly believed that the optimum daily dosage cannot be generalized but must be individualized, as in other drug therapy.

Relapse: Durable clinical results are the prime objective in the successful treatment of tuberculosis and the achievement of this objective, or lack thereof, may be construed as a measure of the effectiveness of a given therapeutic procedure. The increment of relapses over a given period of time is one of the most important criteria of the effectiveness of therapy.

Roentgenological relapse of the disease was determined by the individual investigators in the 332 patients in this series (figure 10). As the percentage of relapse occurring in the four different regimens is cumulative and increases according to the length of time of observation from the beginning of therapy, it should be recalled that the four regimens were evaluated at different periods of time after the beginning of treatment. As patients on regimen D were observed for the longest period of time, the total increment of relapse should be the greatest in this group, unless this regimen was considerably more effective than any of the other three. The results in regimen D were evaluated approximately 100 days later than those in regimen A, and approximately one to two months later than those in regimens C and B. As the percentage of relapse is essentially the same for the four regimens, regimen D appears to be somewhat more effective in the prevention of relapse than the other regimens.

DISCUSSION

Although many problems concerning the most effective use of streptomycin in the treatment of pulmonary tuberculosis remain unsolved or only partially solved, a considerable amount of confirmatory and additional information has emerged since the American Trudeau Society streptomycin project was launched two years ago. Some of this information and better clarified concepts will be discussed.

The objective: The ideal and ultimate objective of an over-all therapeutic program is the securing of lasting arrest of the disease, preservation of adequate pulmonary function, prevention of permanent physical or mental deficiencies, and the restoration to the family and community of an individual who is as capable of carrying on in a competitive society as he was before the development of tuberculosis. The objective of streptomycin therapy must of necessity, however, be more circumscribed and limited. Under certain conditions streptomycin therapy may play only a minor role but in others a vital or decisive role in the over-all therapeutic program. In general and specific terms what should be the primary objective of streptomycin therapy? In general, it may

be considered that the primary objective of streptomycin is to bring into favorable balance vital resistance over susceptibility and dosage of organisms. Specifically, this is brought about by the inhibitory action of streptomycin on the tubercle bacillus.

Several questions immediately arise concerning the possible attainment of the general and specific objectives of streptomycin therapy which are especially important in relation to duration of treatment. (*1*) How rapidly does streptomycin exert a significant antimicrobial effect in clinical tuberculosis? (*2*) Are different degrees of antimicrobial effect obtained? (*3*) What are some of the more important factors that limit streptomycin effectiveness? (*4*) How long *should* effective antimicrobial therapy be maintained? (*5*) How long *can* effective antimicrobial therapy be maintained? (*6*) When and under what circumstances should other therapeutic procedures be used?

With regard to the first question, factors related to the drug, the infecting organism and the patient are all involved. The pharmacology of streptomycin has been thoroughly investigated and is well documented. It is known that the drug is rapidly absorbed into most of the body fluids but to what degree it is concentrated in different types of diseased and damaged tissue is not so well known. Just how rapidly the drug may exert a significant therapeutic effect upon pulmonary tuberculosis is dependent upon a number of factors, the most important of which are the type and extent of the disease. There is considerable evidence to show that therapeutic effect may be exerted quite rapidly under certain circumstances. For instance, rapid defervescence and disappearance of symptoms within forty-eight to seventy-two hours following the initial administration of streptomycin has been observed in patients acutely ill with high fever and other toxemic manifestations of recently developed tuberculous pneumonia. Rapid loss of symptoms referable to laryngeal, bronchial, or intestinal tuberculosis has also been observed within a very short time after the beginning of streptomycin treatment. Probably the most dramatic therapeutic effect of streptomycin is that sometimes noted in patients with tuberculous meningitis. McDermott *et al.* have reported upon the rapid therapeutic effect of streptomycin in both acute generalized lymphohematogenous tuberculosis and tuberculous pneumonia (8, 9).

If the drug has a more rapid and also greater therapeutic effect upon acute disease of recent origin, it could be argued that the duration of treatment for this type of pulmonary disease might be less than that for the subacute or more chronic forms of pulmonary tuberculosis. Moreover, it seems logical to assume that the relative stability of chronic disease is indicative of a more favorable balance between resistance and susceptibility and number of infecting bacilli. The corollary of this is that acute progressive disease is evidence of a relative lack of resistance or the presence of overwhelming dosage of organisms or of both. Rich has pointed out that there is no such thing as "solid immunity" or resistance adequate to prevent spread of the infection provided the dosage of organisms is sufficiently large (10). This fact is frequently demonstrated by the patient with chronic cavernous disease whose sputum is teeming with

tubercle bacilli yet who suffers no spread of disease for long periods of time until a brisk hemoptysis results in the aspiration of an overwhelming dose of bacilli into previously healthy areas of the lung with the development of tuberculous pneumonia. It may be concluded, therefore, that the presence of extensive, acute, predominantly exudative disease indicates a serious imbalance in the host-parasite relationship and is an important factor to be considered in the attainment of the objectives of streptomycin therapy.

Although resistance to tuberculosis can be measured imperfectly at best, it is known that certain patients may have greater actual and potential capabilities of coping with their disease than others. Even though the immediate and particularly the remote potentialities of a given lesion cannot be measured accurately, some idea of the ability of the patient to overcome the disease may usually be estimated. In the final analysis, these fundamental factors of dosage of organisms, patient resistance, the type, extent, and immediate and remote potentialities of the disease, and the possible effective use of other therapeutic procedures serve as guides and principles in the final determination of the proper duration of streptomycin treatment. Thus the objectives of streptomycin therapy may be realized within three or four weeks in some patients, in two to three months in others, and in still others they may never be realized.

Types of disease: The early clinical reports suggesting that recent acute exudative or lobular pneumonic lesions that have not progressed to extensive caseation respond exceedingly well to streptomycin have been confirmed by many investigators, notably those of the Veterans Administration, Army, Navy and the present group. At the January, 1948 meeting of the Chemotherapy Committee, Amberson and Stearns demonstrated illustrative cases of this type showing marked therapeutic effect of streptomycin. The comparative efficacy of streptomycin in different clinical types of disease is well shown in figure 2. Experience has also emphasized the fact that the earlier the drug is used for acute disease the more effective it is in the prevention of irreversible morphologic changes and consequent disturbances of function. It has also been the general experience that, despite rapid clearing of the acute inflammatory process, largely by resolution, many small presumably caseous and partially encapsulated foci remain. These residual foci may be the source of relapse or progression of the disease at some later date, particularly if adequate sanatorium or hospital treatment is not continued for a sufficient period of time.

The large majority of patients with fairly extensive lobular pneumonic disease usually have cavities which may or may not be roentgenologically demonstrable. Probably for this reason, "sputum conversion" without some other treatment in addition to streptomycin, even in patients with acute disease who show striking improvement, is usually not attained with any greater degree of consistency than in patients who have more subacute or chronic types of disease. This is well shown in figure 2.

Streptomycin has proved of great benefit in preparation of patients for thoracoplasty who have extensive exudative disease in addition to cavity formation. It is believed that in many such patients the disease would have continued to

progress and surgery would probably not have become feasible without streptomycin. Several illustrative cases of this type were shown by the group from New York State Tuberculosis Hospitals[7] at the January, 1948 Chemotherapy Committee meeting. Streptomycin has also been found to be of limited or considerable value in preparation of patients for surgery who have predominantly chronic fibrocaseous and cavernous disease with a subacute component and frequently a complicating tuberculous bronchitis. The partial, even though perhaps temporary, control of the more active pulmonary and bronchial disease is often sufficient to permit a relatively safe and effective surgical program. In addition, streptomycin may be of such marked benefit in patients with tuberculous laryngitis or enteritis, with consequent improvement in the general condition, as to allow successful thoracoplasty or other surgical procedures. On the other hand, streptomycin usually has little or no decisive therapeutic value when used alone and directed against chronic types of cavernous disease without recent or exudative components, or in the absence of tuberculous laryngitis, tracheobronchitis or enteritis.

Howlett and O'Connor have made detailed studies of the effect of streptomycin on chronic disseminated nodular tuberculosis of the lungs. At the meeting of the Chemotherapy Committee in January, 1948, they showed illustrative cases and pointed out that streptomycin may be strikingly effective in this type of chronic disease (11).

Methods and frequency of administration: Experience during the past two years has definitely shown that parenteral streptomycin therapy gives the best results. As a result of animal experimentation (6) and later clinical experience it was found unnecessary to administer the drug more often than twice daily, and once daily may be effective for certain patients. The maintenance of a high concentration of streptomycin in the blood is not necessary to achieve beneficial therapeutic results. A given daily dosage administered in four or five injections appears to cause more severe toxic reactions than the same amount administered once or twice daily. Streptomycin given by inhalation or aerosolization is much less effective, even for disease of the mucous membranes of the respiratory tract, than when administered parenterally. Streptomycin given by mouth is usually ineffective and is not recommended.

Daily dosage: Statistical data concerning the relative therapeutic effects of large versus small daily dosages have been presented above. These data would seem to indicate that the larger daily dosages resulted in more frequent as well as more marked improvement than the smaller daily dosages. It should be emphasized, however, that this finding appears to be somewhat at variance with the opinions and experiences of some of the individual investigators, including the writers, whose combined reports constitute the statistical data presented.

[7] Ray Brook State Tuberculosis Hospital, Ray Brook, New York, Dr. Harry A. Bray, Director; Homer Folks Tuberculosis Hospital, Oneonta, New York, Dr. Ralph Horton, Director; Hermann M. Biggs Memorial Hospital, Ithaca, New York, Dr. N. Stanley Lincoln, Director; Mt. Morris Tuberculosis Hospital, Mt. Morris, New York, Dr. Arthur M. Stokes, Director.

In an attempt to clarify this paradoxical finding the sources of probable clinical and statistical error have been sought and an endeavor made to evaluate their effects upon results obtained in the different regimens. Several factors, other than daily dosage, which may influence therapeutic results have already been discussed. Two important factors which significantly influence results in this series of patients are: (*1*) the unequal distribution of similar types of clinical material among the different regimens, and (*2*) the relatively small number of patients on all regimens, but especially on regimen B (46 patients).

Evaluation of the clinical material in each regimen shows that a much larger percentage of patients with predominantly *acute* disease are in regimens B and D than in A and C. The percentage distribution of acute disease in the four regimens is as follows: A, 36.3; B, 60.9; C, 40.4; and D, 52.1 (figure 1). It is generally agreed that the best results from streptomycin are usually attained in patients with recent acute, potentially reversible disease. This fact is strikingly shown in figure 2, in which it is seen that marked therapeutic improvement, measured in terms of clinical, roentgenographic, and over-all improvement, occurred approximately twice as often in patients with acute disease as in those with subacute disease. Moreover, improvement occurred from two and one-half to four times as often in patients with acute disease as in those with chronic disease. It is quite probable, therefore, that the superior results obtained in regimens B and D were more significantly related to the type of clinical material (predominantly acute disease) than to the difference in total daily dosage or the difference in duration of treatment. In this connection it should be recalled that results in regimen C were not as favorable as those in regimen B although patients on the former regimen were treated for the longer period of time. It is reasonable to assume, therefore, that the superior results on regimen B, as compared with those observed in the patients on regimen C, were most probably due to the larger percentage of patients with acute disease on regimen B, and to a less extent to the larger daily dosage given to the patients on this regimen. To recapitulate, it may be said that the most significant factors affecting results in this series of patients are: (*1*) the predominant type of clinical material treated with the individual regimens, (*2*) the total daily dosage, (*3*) the duration of treatment, and (*4*) the emergence of drug-resistant organisms, a phenomenon which is undoubtedly of the greatest significance in limiting the effectiveness of prolonged streptomycin treatment.

Duration of treatment: Before deciding upon the duration of streptomycin therapy for the individual patient, it is essential to consider the objectives, possibilities, and limitations of drug therapy. Effective antibacterial therapy is related to multiple factors, some primarily concerned with the patient, some with the disease, and others with the bacillus and the drug. It is also essential, in considering the duration of streptomycin treatment, to formulate an over-all therapeutic program that effectively integrates streptomycin therapy with other procedures.

If it were possible to eradicate all infection and there were no contraindications, it would be desirable under certain circumstances to continue streptomycin

therapy for whatever time required for the elimination of all viable organisms. As this is not possible and as there are a number of factors limiting the duration of effective therapy, the period of treatment selected for different types of pulmonary tuberculosis must be a compromise. The best compromise can be ascertained only after establishing a definite objective and careful weighing of all factors involved, immediate, remote and potential.

Emergence of streptomycin-resistant tubercle bacilli: Once the forces of vital resistance have become favorably balanced over dosage of organisms as a result of streptomycin therapy, the question arises as to how long should streptomycin be continued. If "drug-fast" organisms did not replace sensitive organisms in proportion to the duration of treatment and provided the drug produced no toxic effects, it probably would be wise to continue its use for an indefinite period of time on the principle that other therapeutic measures are used, namely, until the disease is well arrested. By adjusting the dosage of streptomycin on a basis of milligrams per kilogram of body weight, serious toxic effects usually can be avoided. It is not possible at present, however, to prevent the emergence of resistant strains of organisms, although there is some evidence to suggest that this occurrence may be delayed or reduced as a result of the simultaneous use of combined chemotherapy. It also appears that organisms become "drug-fast" regardless of the method or frequency of administration or the total daily dosage given. It has been well established that "drug-fastness" is definitely related to duration of treatment and that the incidence of resistant strains increases with prolongation of treatment. Organisms rarely become "drug-fast" before the end of the first month of streptomycin therapy. Usually, however, 75 to 80 per cent of patients discharge significantly "drug-fast" organisms by the end of the third or fourth month of treatment.

As streptomycin is usually essentially ineffective after the organisms become "drug-fast" and in view of the fact that pulmonary tuberculosis is characteristically a relapsing disease, the tremendous importance of avoiding the emergence of significant numbers of "drug-fast" organisms is obvious. If the primary objectives and limitations of streptomycin are kept in mind, and provided proper indications are observed and other therapeutic procedures are used at the opportune time, it is believed that the duration of streptomycin treatment may usually be sufficiently limited to avoid "drug-fast" organisms. There are certain exceptions to this statement. For example, it may be necessary to continue streptomycin for longer than four or six weeks for acute bilateral tuberculous pneumonia or for acute disseminated lymphohematogenous disease (nonmiliary) and under certain other circumstances in which there is no other effective therapy. Under such circumstances more prolonged treatment or retreatment seems justified, regardless of the emergence of some drug-fast organisms. The drug may continue to be of some limited value even though the majority of the infecting bacteria have become "fast." This reasoning is based upon the fact that scattered colonies of sensitive organisms are occasionally found in the presence of a large majority of resistant cells, or the reverse may be true.

It may be concluded, therefore, that optimum duration of treatment, just

as optimum dosage, is dependent upon weighing all factors involved, and in the final analysis must be decided upon in the light of the needs of the individual patient. This decision can best be made at the bedside.

Toxic effects: When given in relatively large daily dosage over prolonged periods, the toxic effects of streptomycin are second only in importance to the emergence of drug-fast organisms as a limiting factor in the usefulness of the drug in the treatment of tuberculosis. The early experiences (2, 7) showed that, when streptomycin was administered intramuscularly in 2.0 to 3.0 Gm. daily doses, it frequently caused disturbing and often severe toxic manifestations, the most serious being deafness and loss of labyrinthine function. Many other usually less significant reactions of a wide variety have also been observed.

As a result of the above findings, the present group explored many dosage schedules in an attempt to discover therapeutically effective but essentially nontoxic regimens. Daily dosage schedules ranging from 0.5 Gm. to 3.0 Gm. were tested. As mentioned above, few patients in the series received as much as 2.0 to 3.0 Gm. daily and the great majority received only 1.0 to 1.5 Gm. daily. With the smaller daily doses, the incidence and severity of toxic manifestations were significantly less. Deafness was not reported in any of the patients treated for pulmonary tuberculosis who are included in this report. Varying degrees of vestibular dysfunction, as evidenced by caloric stimulation tests and clinical symptoms or both, were observed fairly often. It should be mentioned that periodic caloric tests may show the presence of considerable depression of the labyrinthine function in the absence of symptoms. Depression of the labyrinthine function may disappear during treatment and it occasionally disappears after completion of treatment. Moreover, in patients having complete loss of labyrinthine function, the compensatory balancing mechanisms usually become sufficiently effective to prevent prolonged and marked disability. Although such patients may continue to improve for several months, difficulty in walking in the dark may persist indefinitely.

Serious toxic manifestations can be largely avoided or greatly minimized if careful attention is paid to the selection of the proper dosage schedule, which is best calculated on a milligram per kilogram body weight basis. In addition, periodic clinical and laboratory observations should be made, including caloric stimulation tests, audiograms and blood urea nitrogen determinations when indicated. Finally, toxic effects of any consequence are rarely encountered in patients receiving a daily dosage of 20 mg. per kg. of body weight. Although this dosage is undoubtedly beneficial in the majority of patients when proper indications are observed, nevertheless there is considerable evidence to suggest that a daily dosage of 30 to 40 mg. per kg. of body weight perhaps exerts a greater therapeutic effect, at least in patients with certain types of disease. As dihydrostreptomycin appears to be of lower toxicity than streptomycin in daily dosages of 30 to 40 mg. per kg. of body weight, it is believed that it may be possible to provide greater antimicrobial effect with the derivative than with the parent drug (12). In any event, it has been conclusively demonstrated that therapeutically effective and essentially nontoxic dosage schedules of streptomy-

cin can be determined with considerable accuracy for the types of pulmonary tuberculosis which usually respond favorably to the drug.

Relapse: There is no theoretical reason to assume, or experimental, pathological or clinical evidence to indicate, that streptomycin, especially when used without collapse therapy, should or will reduce the incidence of relapse. On the contrary, it is logical to believe that the opposite may prove to be true. If the term relapse is used in a broad sense to denote progression of disease that has become clinically or roentgenologically stabilized or regressive as a result of streptomycin therapy, or the reactivation of disease that has become arrested, there is considerable reason to believe that relapse is more likely to occur in patients who have received streptomycin. The reasons for this belief are: (*1*) Many patients who do not receive streptomycin will not improve and therefore will not "qualify" or become candidates for relapse for their disease may steadily progress until death ensues. (*2*) Conversely, many patients who receive streptomycin will improve without attaining closure of cavities or reversal of infectiousness. In such instances the disease will usually relapse, particularly if collapse therapy or some other definitive type of treatment is not used at the opportune time. (*3*) Patients who improve without streptomycin do so because of the re-establishment of a favorable host-parasite relationship as a result of an autogenous mechanism. Patients who improve as a result of autogenous mechanisms are more likely to attain lasting results than are patients who have inferior resistance and improve as a result of streptomycin therapy, an exogenous mechanism. (*4*) There is no evidence to indicate that streptomycin, even when it produces striking improvement, increases the native resistance of the patient, an important factor in the incidence of relapse.

For these and other reasons previously mentioned which clearly indicate that streptomycin is usually not a definitive form of therapy, additional treatment at the opportune time is essential if relapse is to be minimized or avoided. Relapse may occur (*a*) during treatment, (*b*) soon after completion of treatment, or (*c*) with the emergence of resistant organisms. Therefore, it is evident that, if collapse therapy or other surgery is to be used most effectively and under the most favorable circumstances, it must be instituted in many instances within a few weeks after streptomycin is begun. Several of the present group of investigators have reported that many patients with extensive and recently acute, predominantly exudative disease have been able to have extensive surgery with remarkably little difficulty and with excellent clinical and bacteriological results.

SUMMARY

The results of streptomycin therapy in 332 patients with pulmonary tuberculosis, treated by a group of cooperating investigators in the American Trudeau Society, are reported in summary form.

On the basis of clinical, roentgenologic, and bacteriologic observations, it was clearly demonstrated, as previously reported by others, that the degree of therapeutic efficacy of streptomycin is definitely related to the *character* of the pulmonary disease. Although improvement occurred much more frequently

and was more marked in patients with predominantly acute disease than in those with predominantly chronic disease, nevertheless significant improvement was reported by most of the investigators in a small percentage of patients with chronic disease. As the progression of *chronic* tuberculosis is fundamentally related to the occurrence of *acute* episodes, the great potentialities of the strategic use of short courses of streptomycin to combat such episodes should be stressed.

The larger daily dosages of streptomycin appeared to bring about more frequent and more marked improvement than the smaller daily dosages. Even though the larger daily dosages appeared to produce greater therapeutic effect, this advantage was often minimized or nullified by the more severe toxic manifestations incident to larger dosage. The availability of dihydrostreptomycin, which appears to be less neurotoxic than streptomycin, may aid in the solution of this problem.

The occurrence of drug-fast organisms was found to be definitely related to duration of treatment. Organisms resistant to 10 or more γ of streptomycin rarely occurred in patients treated for one month, but appeared frequently in patients treated for two months or more. It appears that "drug-fastness" is unquestionably a significant factor in limiting the effectiveness of the antibiotic.

The failure to close cavities and convert sputum in a larger percentage of patients and the relapse of the disease with increasing frequency with passage of time (patients observed six to ten months after the beginning of treatment) suggest the limitations of antibacterial therapy for this characteristically chronic disease and emphasize the absolute necessity of also using more definitive treatment, notably collapse therapy, if lasting results are to be achieved.

SUMARIO

Obra de Investigación de la Estreptomicina en la Tuberculosis por la American Trudeau Society. Memoria Sumarizada

Esta memoria sumariza los resultados de la estreptomicinoterapia en 332 tuberculosos pulmonares tratados en cooperación por un grupo de investigadores de la American Trudeau Society.

A base de las observaciones clínicas, radiológicas y bacteriológicas, demostróse claramente, según ya han comunicado otros autores, que la eficacia terapéutica de la estreptomicina guarda relación bien definida con la *naturaleza* de la afección pulmonar. Aunque la mejoría fué mucho más frecuente y más pronunciada en los casos predominantemente agudos que en los predominantemente crónicos, la mayor parte de los investigadores comunicaron también mejoria significativa en un pequeño porcentaje de los enfermos crónicos. Como la agravación de la tuberculosis *crónica* se relaciona fundamentalmente con la ocurrencia de episodios *agudos*, hay que recalcar las grandes potencialidades que entraña el empleo estratégico de breves series de estreptomicina para combatir dichos episodios.

Las dosis diarias mayores de estreptomicina obtuvieron al parecer mejorías más frecuentes y más notables que las dosis diarias más pequeñas. Si bien las primeras produjeron aparentemente mayor efecto terapéutico, esta ventaja

fué a menudo atenuada o borrada por las manifestaciones tóxicas mas graves que las acompañaron. En la solución de este problema tal vez ayude la introducción de la dihidroestreptomicina, que parece ser menos tóxica que la estreptomicina.

La aparición de microbios estreptomicinorresistentes resultó hallarse netamente relacionada con la duración del tratamiento. Microbios resistentes a 10 o más γ de estreptomicina fueron raramente observados en los enfermos tratados por un mes, pero aparecieron frecuentemente en los tratados por dos meses o más. Según parece, la farmacorresistencia constituye indudablemente un factor importante en la limitación de la efectividad del antibiótico.

El fracaso en lo relativo a clausura de las cavernas y viraje del esputo en un porcentaje mayor de enfermos y las recidivas cada vez más frecuentes con el transcurso del tiempo (en los sujetos observados de seis a diez meses desde el comienzo del tratamiento) indican las limitaciones de la antibacterioterapia en esta enfermedad tan típicamente crónica y recalcan la necesidad abosulta de usar además un tratamiento más definitivo, notablemente la colapsoterapia, si se van a lograr resultados duraderos.

Acknowledgments

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CONSTITUTIONAL FACTORS IN RESISTANCE TO INFECTION[1, 2]

I. The Effect of Estrogen and Chorionic Gonadotropin on the Course of Tuberculosis in Highly Inbred Rabbits

MAX B. LURIE, SAMUEL ABRAMSON[3] AND MARVIN J. ALLISON

Introduction

In a study (1) on the native resistance of inbred rabbit families of varying hereditary resistance to tuberculosis it was found that the fundamental variant in the disease developed by these families was the degree of localization of the infection at the portal of entry. Families of high resistance effectively limited the process to the lung, if the tuberculosis was of natural respiratory origin, with little or no dissemination of the infection by hematogenous or lymphogenous routes. In families of low resistance, on the other hand, the primary tuberculosis in the lung progressed rapidly and was soon widely disseminated through the body by the vascular system. As bacterial infection takes place chiefly in the connective tissue, it was thought that the permeability of this tissue to particulate matter might be one of the factors in this resistance. In fact, in some families there is a parallelism between the spread of tuberculosis and the spread of India ink in the connective tissue of the skin. That the spread of particulate matter in the skin might be under the influence of naturally occurring hormones was suggested by the observation that male rabbits (2) limited this spread more effectively than females. Furthermore, Sprunt (3) has shown that the administration of estrogen restricted the spread of India ink in the skin. On the other hand, the intravenous injection of chorionic gonadotropin (4), which induced the formation of corpora lutea in the ovaries and the secretion of progesterone, enhanced this spread.

It would follow, therefore, if the spread of particles, and among them tubercle bacilli, is a factor in the native resistance to tuberculosis, that suitable treatment of animals with estrogen should retard the tuberculous process. Conversely, appropriate exposure of rabbits to chorionic gonadotropin or progesterone should enhance the disease.

In this and the subsequent studies an endeavor was made to test this assumption. In general, the observations made substantiate the expected results. Analysis of the results, however, has led beyond the point of departure of the effect of these hormones on the spread of particles to the problem of their influence on the tuberculin sensitivity of the skin and on the allergy of the internal organs; to their action on the irritability of the skin to noxious agents in general; and to their role in the occurrence of amyloid degeneration. The effect of these hormones on the circulating lymphocytes, on the adrenal, and on antibody

[1] From The Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Aided by a grant from the Commonwealth Fund.

[3] Tuberculosis Control Division, Public Health Service, Federal Security Agency.

production was studied with the view of a possible interrelation between these hormones and the functions of the pituitary gland and the adrenal cortex.

Materials and Methods

Female litter mate pairs of the susceptible family C (1) of the same age and of identical genetic and environmental history for the past six to eight inbred generations were divided into two groups. One-half served as controls and received 0.5 cc. of sterile sesame oil subcutaneously once a week. The other litter mates were given 0.5 mg. α of estradiol dipropionate[4] in 0.5 cc. sesame oil at the same time and by the same route. Rabbit pairs older than 10 months were ovariectomized, divided into control and experimental groups, and treated in the same manner. After the experimental rabbits had been under the influence of the estrogen for about two weeks, 0.5 cc. of 1:5 dilution of autoclaved India ink in saline was injected intracutaneously into both groups. The spread of the particulate matter in the skin was determined on the next day by planimeter in a manner previously described (4). The results will be discussed in the last paper of this series. When the estrogenic effect in the experimental animals was indicated by a marked increase in the size and congestion of the vulva and a definite enlargement of the nipples, these rabbits, together with their litter mate controls, were infected intracutaneously with the same dose of virulent bovine tubercle bacilli of the Ravenel strain. The inoculations were made on one side of the chest wall at the same distance from the midline and from the axilla. As the number of suitable litter mate pairs from these inbred families available at any one time was small, it was necessary to limit each individual experiment to 4 paris and to repeat the experiment three times. The infecting dose varied in each group and was determined by colony counts of the inoculum (5). Needless to say, in each experiment the control and estrogen-treated rabbits received the same dose. The estrogen and sesame oil injections, respectively, were continued at weekly intervals throughout the course of the disease.

Two additional similar experiments were performed with litter mate pairs of the ninth and tenth inbred generations of the more resistant family A. The special conditions obtaining in these will be detailed later.

Two and seven days after inoculation, and at weekly intervals thereafter until the death of the animal, the skin lesion at the site of infection was measured and its volume determined according to a formula previously described (1). Likewise, the size of the axillary lymph nodes draining the site of inoculation was followed throughout the course of the disease.

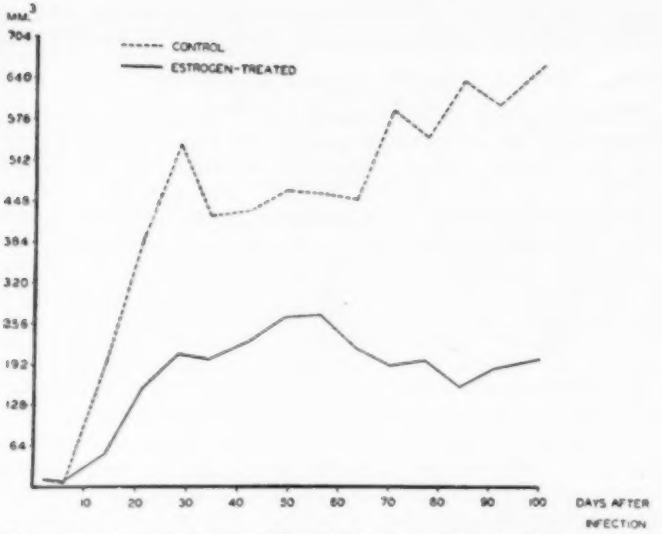
Whenever one of a pair died, its litter mate was killed and the extent of the disease in the draining lymph nodes and lungs was determined objectively by their weight in grams. A careful search of all the organs was made for disseminated lesions and their character and magnitude was estimated. The weight of the uterus and vagina, the adrenals, the pituitary and other organs was determined.

Observations

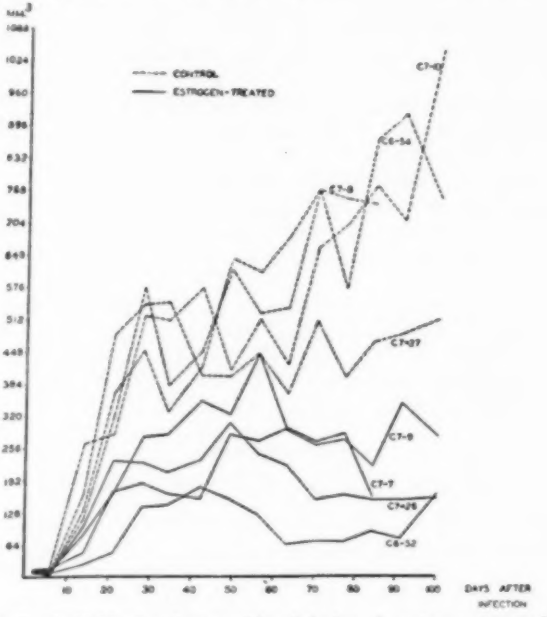
The effect of estrogen on tuberculosis

In text figure 1 may be seen the average volume of the lesion at the site of inoculation in the skin throughout the course of the disease in estrogen-treated

[4] This and other sex hormones were generously furnished by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc.



TEXT FIGURE 1. Average volume of local lesion in estrogen-treated and control litter mates of the C family (Experiment of 1944–1945).



TEXT FIGURE 2. Individual volumes of local lesions in estrogen-treated and control litter mates of the C family (Experiment of 1944–1945).

and control litter mates of the first experiment on the C family. The infecting dose was 82,000 viable bacilli. In text figure 2 is plotted the individual volumes of these lesions in each of these 8 rabbits during the entire course of the infection. It is evident that the progress of the lesion at the site of inoculation is markedly and uniformly retarded in the estrogen-treated rabbits as compared with that in their litter mate controls.

TABLE 1

The effect of estrogen on the progress of tuberculosis in litter mate pairs of the inbred, susceptible family C; experiment 1944–1945

CONTROL, C OR EXPERIMENTAL, E	RABBIT NUMBER	PARENTS AND AGE	DIED D, KILLED K	VOLUME OF LESION AT SITE OF INOCULATION ON 28TH DAY AFTER INFECTION	WEIGHT OF UTERUS AND VAGINA	WEIGHT OF TUBERCULOUS NODES DRAINING SITE OF INFECTION	EXTENT OF DISEASE IN LUNGS AND THEIR WEIGHT	EXTENT OF DISEASE IN PLEURA	EXTENT OF DISEASE IN KIDNEYS	NUMBER OF OTHER ORGANS METASTASIZED
		months		*mm.*³**		*grams*	*grams*			
C	C6-54*	C5-52 x C5-40 10.6	D	578	6.0	1.8	+++ 39.7	+++	++	2
E	C6-52*	C5-52 x C5-40 10.6	K	140	36.6	2.1	+ 12.3	±	±	1
C	C7=27	C5-52 x C6-40 4.0	D	544	9.3	4.7	++++ 52.1	++±	+++	8
E	C7=26	C5-52 x C6-40 4.0	K	228	16.7	2.5	++ 19.1	+±	++	4
C	C7-8	C6-11 x C6-40 4.0	K	452	7.2	4.7	++++ 47.3	++	+++	6
E	C7-7	C6-11 x C6-40 4.0	D	187	13.6	1.2	+++ 43.5	++	++	2
C	C7-10	C6-11 x C6-40 4.0	K	520	5.8	4.7	+++ 38.8	++++	+++	10
E	C7-9	C6-11 x C6-40 4.0	K	280	19.8	2.3	+++ 33.2	+++	+++	6

* Ovariectomized on sixth and seventh day following infection.
** Per cubic centimeter of free flowing venous blood.

In table 1 are presented the essential data at the time of death, which, it will be remembered, was the same for the two rabbits of each pair. In column 3 the parents and age of each of these pairs are recorded. They are identical in each instance. The volume of the lesion at the site of inoculation on the twenty-eighth day of infection, listed in column 5, again shows how uniformly the growth of the lesion in the estrogen-treated rabbits was retarded. That the estrogen exercised its physiological effect on the experimental animals is shown in column 6, where the weight of the uterus and vagina of each of the estrogen-treated rabbits is seen to be approximately two to six times that of its litter mate con-

trol. The extent of the disease in the lymph nodes draining the site of inoculation and the tuberculosis in the lungs in general is less in the experimental than in the control animals. The same is true for the remaining organs. In each case, however, the degree of retardation varies; in some it is marked, in others it is less pronounced. The most consistent observation in this and in subsequent experiments of the same character appears in the last column where it may be seen that the number of metastases to other organs is uniformly less in the estrogen-treated than in their control litter mates. It may be noted that ovariectomy did not diminish the retarding effect of estrogen on the progress of the disease.

In text figure 3 may be seen the average progress of the lesion at the site of inoculation in 4 estrogen-treated rabbits and in 4 litter mate controls of the second experiment on the C family, where the infecting dose contained 162,000 living microorganisms. Again it is clear that estrogen markedly retarded the growth of the lesion at the portal of entry throughout the course of infection. Figures 1 and 2 (Plate I) illustrate this effect in the two ovariectomized litter mates, C7-13 and C7-12, during the fourteenth week of the infection. The larger size of the nipple of the latter attests to the estrogenic influence to which it was subjected.

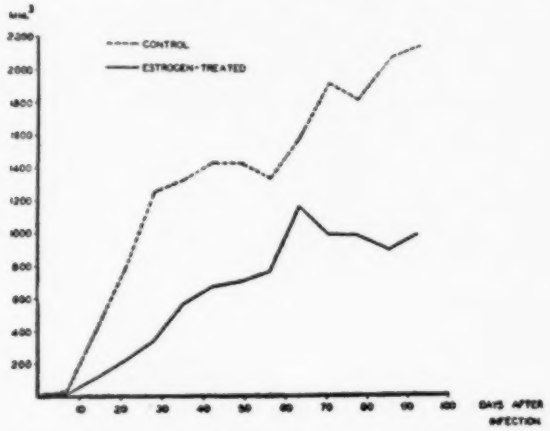
Table 2 presents in detail the extent of tuberculosis in the lung and in the other organs at the time of death, as well as other pertinent data listed in the previous experiment. It is noteworthy that all of the control rabbits died, whereas all the estrogen-treated mates were killed. Figures 5, 6, 7 and 8 (Plate II) depicit the tuberculosis in the lung and kidney and the development of the uterus and vagina in each of these 4 pairs at autopsy. It is clear that in every instance where the rabbit was under the influence of estrogen, as indicated by the larger size of the uterus, the disease was less extensive than in its litter mate control. Again the retarding effect of estrogen was pronounced in some and less marked in others. As in the last experiment, the number of organs metastasized was conspicuously reduced in the estrogent-reated animals.

In an effort to determine whether estrogen treatment will effectively suppress an infection with small numbers of virulent tubercle bacilli, a third group of 4 litter mate pairs of the susceptible family C was inoculated intracutaneously with about 10 virulent tubercle bacilli, as determined by culture. The progress of the lesion at the site of inoculation in the control and estrogen-treated rabbits is presented in text figure 4.

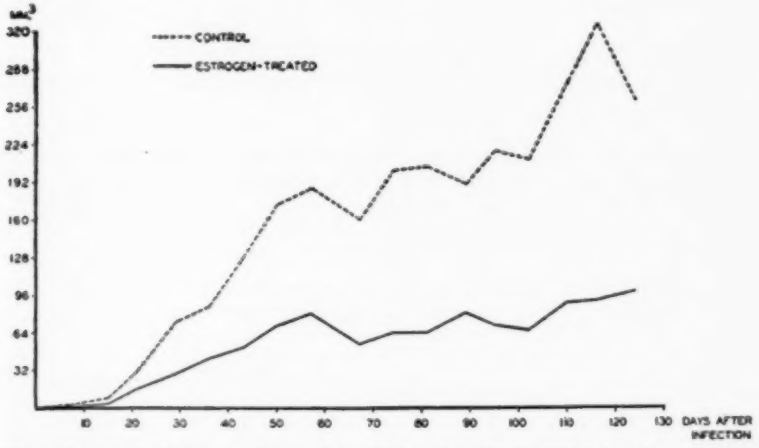
The striking effect of bacillary dosage on the rate of growth of the cutaneous lesion both in the control and estrogen-treated rabbits is evident by comparing the progress of the lesions in this group with that in the two previous experiments as recorded in text figures 1 and 3, respectively. The delay in the development of the lesion with the smaller dose is pronounced. Nevertheless despite this very slow development of the disease, estrogen treatment only retarded its progress, but did not suppress it.

The results are presented in detail in table 3. It may be noted that in 3 of the 4 pairs estrogen definitely slowed the progress of the disease in the internal

organs and reduced its dissemination. In one of these pairs, however, the tuberculosis in the estrogen-treated litter mate was actually more extensive than in the control. It is noteworthy that this pair was quite young and had not



TEXT FIGURE 3. Average volume of local lesion in estrogen-treated and control litter mates of the C family (Experiment of 1945–1946).



TEXT FIGURE 4. Average volume of local lesion in estrogen-treated and control litter mates of the C family (Experiment of 1946–1947).

attained sexual maturity at the beginning of the experiment. While the uterus and vagina of all the estrogen-treated rabbits were quite hypertrophied, these organs were also quite large in some of the control litter mates. This is possibly

TABLE 2

The effect of estrogen on the progress of tuberculosis in litter mate pairs of the inbred, susceptible family C; experiment 1945-1946

CONTROL, C OR EXPERIMENTAL, E	RABBIT NUMBER	PARENTS AND AGE	DIED D, KILLED K	VOLUME OF LESION AT SITE OF INOCULATION ON 28TH DAY AFTER INFECTION	WEIGHT OF UTERUS AND VAGINA	WEIGHT OF TUBERCULOUS NODES DRAINING SITE OF INFECTION	EXTENT OF DISEASE IN LUNGS AND THEIR WEIGT	EXTENT OF DISEASE IN PLEURA	EXTENT OF DISEASE IN KIDNEYS	NUMBER OF OTHER ORGANS METASTASIZED
		months		mm.³**		* *grams*	*grams*			
C	C7-36	C6-11 x C6-40 4.8	D	1,360	6.3	2.5	+++++ 67.6	++	++++	16
E	C7-40	C6-11 x C6-40 4.8	K	408	22.2	3.75	++± 22.5	+++	+++	8
C	C7-16*	C6-11 x C6-40 12.9	D	926	7.9	7.3	+++++ 74.4	++	++±	4
E	C7-15†	C6-11 x C6-40 12.9	K	486	20.7	1.95	+++ 43.5	+	+	3
C	C7-29	C6-11 x C6-40 7.3	D	1,544	7.2	0.96	++± 27.2	++±	++	8
E	C7-28	C6-11 x C6-40 7.3	K	345	25.7	1.07	± 10.3	+++	+	2
C	C7-13*	C6-11 x C6-40 12.9	D	1,207	6.5	3.55	+++++± 56.5	+++++	+++	7
E	C7-12*	C6-11 x C6-40 12.9	K	240	27.8	3.35	++ 20.0	+	+±	4

* Ovariectomy performed three weeks before inoculation.

† Rabbit had long standing infection of subcutaneous tissues which persisted for 70 days during the course of the infection.

** Per cubic centimeter of free flowing venous blood.

accounted for by the fact that these females were inadvertently exposed to males fourteen to nineteen days prior to the determination of their uterine weights.

A fourth trial with estrogen was made on 4 litter mate pairs of the tenth in-

PLATE I

FIG. 1. (Upper left). Lesion at site of inoculation in skin of control rabbit C7-13 on fourteenth week of infection. The large ulcerated lesion is seen in upper half of photograph with extension toward the abdomen below it. The large draining axillary node is seen in the left upper portion of the figure. The nipple is below the label and to the left.

FIG. 2 (Upper right). Lesion at site of inoculation in skin of estrogen-treated rabbit C7-12 (litter mate of C7-13, shown in figure 1), on the fourteenth week of infection. A small lesion with little spread is seen in the lower half of the figure. Compare the size of the nipple in this rabbit, seen above the ulcer, with that of its litter mate in figure 1.

FIG. 3 (Lower left). Lesion at site of inoculation in skin of control rabbit A9 = 54 on the 128th day of infection. The well-defined, raised lesion with its small ulcer is seen in the upper left portion of the photograph.

FIG. 4 (Lower right). Lesion at site of inoculation in skin of gonadotropin-treated rabbit A9 = 56 (litter mate of A9 = 54, shown in figure 3) on the 128th day of infection. The large ulcer, with numerous metastases in the skin, and the extensive involvement of the draining lymph nodes are readily seen.

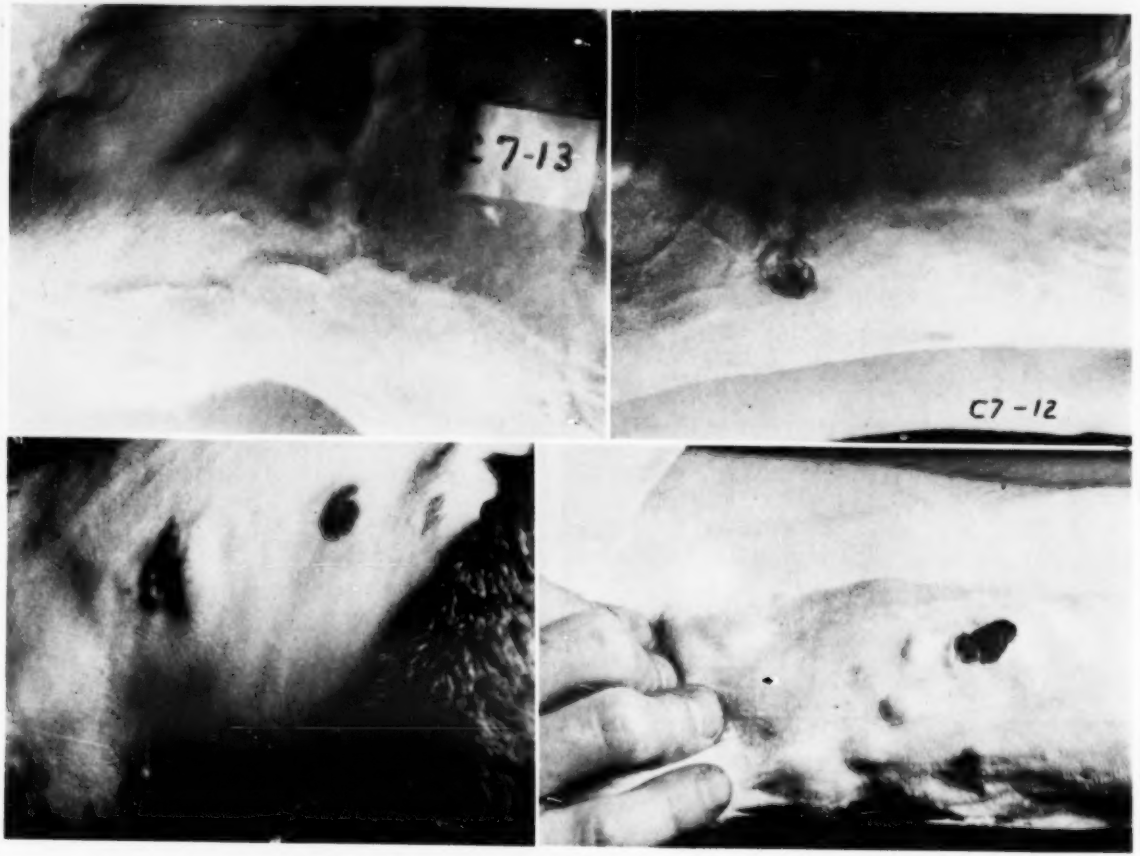


PLATE I

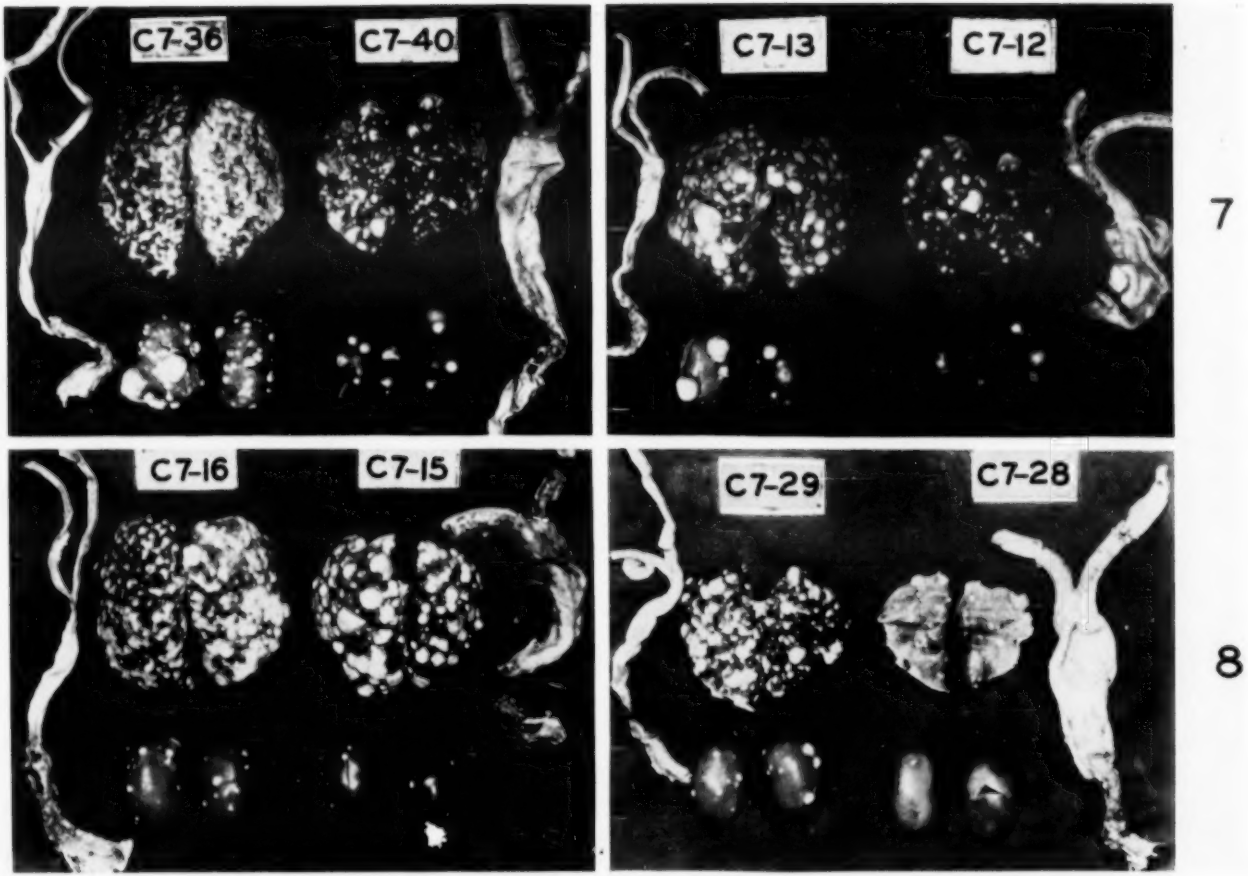


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PLATE II

TABLE 3

The effect of estrogen on the progress of tuberculosis in litter mate pairs of the inbred, susceptible family C; experiment 1946–1947

CONTROL, C OR EXPERIMENTAL, E	RABBIT NUMBER, PARENTS AND AGE, IN MONTHS	DIED D, KILLED K	VOLUME OF LESION AT SITE OF INOCULATION ON 57TH DAY AFTER INFECTION	WEIGHT OF UTERUS AND VAGINA	WEIGHT OF TUBERCULOUS NODES DRAINING SITE OF INFECTION	EXTENT OF DISEASE IN LUNGS AND THEIR WEIGHT	EXTENT OF DISEASE IN PLEURA	EXTENT OF DISEASE IN KIDNEYS	NUMBER OF ORGANS METASTASIZED BEYOND SITE OF INOCULATION
			*sq.mm.***	*grams*	*grams*	*grams*			
C	C8-2* C7-5 x C6-40, 12.8	D	378	0.7	3.5	+++++ 71.5	+++	++++	13
E	C8=1* C7-5 x C6-40, 12.8	K	125	29.7	1.9	+++ 39.4	+±	++±	11
C	C8=17 C7-38 x C6-37, 5.8	K	168	20.6	5.1	++ 16.9†	+++	++	12
E	C8=16 C7-38 x C6-37, 5.8	K	66	21.9	0.8	++ 16.1	++	±	7
C	C8=19 C7-38 x C6-37, 5.8	K	106	13.0	0.7	++± 22.8	+	+	6
E	C8=18 C7-38 x C6-37, 5.8	K	79	18.6	1.9	± 9.7	+±	+	4
C	C8=23 C7-43A x C6-37, 4.2	K	96	17.4	1.3	± 9.2	±	±	4
E	C8=24 C7-43A x C6-37, 4.2	K	50	19.5	0.8	++ 17.5	+	++±	6

* Ovariectomized.

† The total number of tubercles in the lung is far greater than that in the litter mate C8=16.

** Per cubic centimeter of free flowing venous blood.

bred generation of the more resistant family A. The dose of estrogen was reduced to 0.125 mg. α estradiol dipropionate twice weekly in 0.5 cc. sesame oil subcutaneously. The control litter mates received two weekly injections of 0.5 cc. sesame oil without the estrogen. Two were male pairs and two were females. The inoculum for intracutaneous infection contained 220,000 virulent bacteria.

The observations are presented in text figure 5 and table 4. It is clear that

PLATE II

FIG. 5 (Upper left). Tuberculosis in the lungs and kidneys, and the state of the uterus and vagina in control rabbit C7-36 and estrogen-treated litter mate rabbit C7-40. (Experiment of 1945–1946.)

FIG. 6 (Lower left). Tuberculosis in the lungs and kidneys, and the state of the uterus and vagina in control rabbit C7-16 and estrogen-treated litter mate rabbit C7-15. (Experiment of 1945–1946.)

FIG. 7 (Upper right). Tuberculosis in the lungs and kidneys, and the state of the uterus and vagina in control rabbit C7-13 and estrogen-treated litter mate rabbit C7-12. (Experiment of 1945–1946.)

FIG. 8 (Lower right). Tuberculosis in the lungs and kidneys, and the state of the uterus and vagina in control rabbit C7-29 and estrogen-treated litter mate rabbit C7-28. (Experiment of 1945–1946.)

the retarding effect of estrogen on the growth of the lesion at the site of inoculation is again confirmed. In all but one of the pairs the extent of the disease in the lungs and the number of metastatic foci were less in the estrogen-treated

TABLE 4

The effect of estrogen on the progress of tuberculosis in litter mate pairs of inbred family A; Experiment 1946–1947

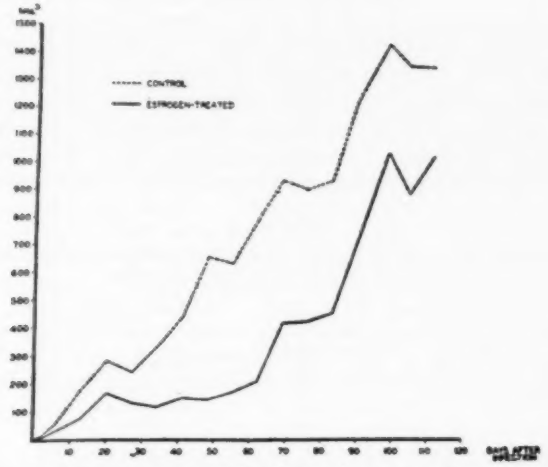
CONTROL, C OR EXPERIMENTAL, E	RABBIT NUMBER AND SEX, PARENTS, AGE IN MONTHS AND WEIGHT IN GRAMS AT BEGINNING OF EXPERIMENT	DIED D, KILLED K	VOLUME OF LESION AT SITE OF INOCULATION ON 41ST DAY AFTER INFECTION	WEIGHT OF UTERUS AND VAGINA	WEIGHT OF TUBERCULOUS NODES DRAINING SITE OF INFECTION	EXTENT OF DISEASE IN LUNGS AND THEIR WEIGHT	EXTENT OF DISEASE IN PLEURA	EXTENT OF DISEASE IN KIDNEYS	NUMBER OF ORGANS METASTASIZED BEYOND SITE OF INOCULATION
			mm.³**		*grams*	*grams*			
C	A10=19 ♂ A9=86 x A8=99 6 months; wt. 2,750 Gm.	D	823	—	5.4	+++ 36.6	++++	++±	15
E	A10=16 ♂ A9=86 x A8=99 6 months; 2,850 Gm.	K	112	—	3.3	+± 16.2	+±	+	5
C	A10=23 ♂ A8=86 x A8=99 3.3 months; 2,250 Gm.	K	202	—	14.3	+± 17.0	±	±	8
E	A10=21 ♂ A9=86 x A8=99 3.3 months; 1,850 Gm.	D	150	—	—	+++± 43.7	+++	++	11
C	A10=26 ♀ A9=125 x A8=99 4 months; 2,650 Gm.	D	320	7.8	—	+++ 31.7	0	±	13
E	A10=25 ♀ A9=125 x A8=99 4 months; 2,400 Gm.	K	151	12.2	8.5	++± 27.3	++	++	11
C	A10=30 ♀ A9=125 x A8=99 4 months; 2,450 Gm.	D	420	7.7	3.0	+++++ 60.1	++	++++	11
E	A10=27 ♀ A9=125 x A8=99 4 months; 2,250 Gm.	K	179	30.7	2.35	++++ 57.6	+	++	7

** Per cubic centimeter of free flowing venous blood.

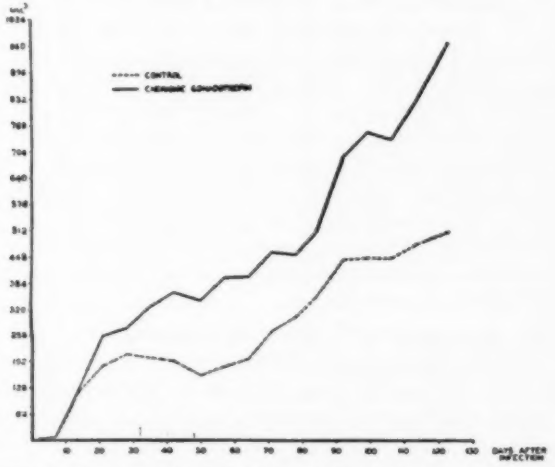
animals. The single litter mate in which the estrogen failed was only 3.3 months old at the beginning of the experiment.

The results of the four experiments are summarized in table 5. The pairs are listed in order of the age of the rabbits at the beginning of the experiment. The extent of the disease in the lung, pleura and kidney is given a single numerical

value, which is the sum of the degree of "plus" involvement of each, one plus being assigned the value of one.



TEXT FIGURE 5. Average volume of local lesion in estrogen-treated and control litter mates of the A family (Experiment of 1946).



TEXT FIGURE 6. Average volume of local lesion in chorionic gonadotropin-treated and control litter mates of the A family (Experiment of 1943).

It is clear that estrogen retarded the progress of the disease at the site of inoculation in the skin of all rabbits. In 13 out of the 16 pairs tested the estrogen definitely reduced the extent of the disease in the lung, pleura and kidney, which

are the usual seats of the most extensive lesions, and diminished the number of foci disseminated by lymphogenous and hematogenous routes. Ovariectomy did not influence this beneficial effect of estrogen. In one pair aged 4 months, while the number of metastases was reduced in the estrogen-treated mate, the extent of the disease in the above organs was greater. In two pairs aged 3.3 and 4.2 months, respectively, estrogen appeared to exert an enhancing effect on the extent and dissemination of the disease. Furthermore, in 12 of these pairs, the experiment was continued until the death of one of the litter mates. The remaining 4 pairs were killed. It is noteworthy that in the first group 10 of the control rabbits succumbed before the estrogen-treated mate. Only two experi-

TABLE 5

The effect of estrogen on the progress of tuberculosis in litter mate pairs according to their age at the beginning of the experiment

CONTROL					ESTROGEN-TREATED				
Litter mate pair	Age at beginning of experiment	Extent of disease in lung, pleura and kidney	Number of metastic foci	Killed K, died D	Litter mate pair	Age at beginning of experiment	Extent of disease in lung, pleura and kidney	Number of metastatic foci	Killed K, died D
	months					months			
(1)	12.9*	9.5	4	D	(1)	12.9*	5.0	3	K
(2)	12.9*	12.5	7	D	(2)	12.9*	4.5	4	K
(3)	12.8*	12.0	13	D	(3)	12.8*	7.0	11	K
(4)	10.6*	8.0	2	D	(4)	10.6*	2.0	1	K
(5)	7.3	7.0	8	D	(5)	7.3	4.5	2	K
(6)	6.0	9.5	15	D	(6)	6.0	4.0	5	K
(7)	5.8	7.0	12	K	(7)	5.8	4.5	7	K
(8)	5.8	4.5	6	K	(8)	5.8	3	4	K
(9)	4.8	11.0	16	D	(9)	4.8	8.5	8	K
(10)	4.2	1.5	4	K	(10)	4.2	5.5	6	K
(11)	4.0	11.0	11	D	(11)	4.0	7.0	7	K
(12)	4.0	9.5	8	D	(12)	4.0	5.5	4	K
(13)	4.0	9.0	6	K	(13)	4.0	7.0	2	D
(14)	4.0	10.0	10	K	(14)	4.0	9.0	6	K
(15)	4.0	3.5	13	D	(15)	4.0	6.5	11	K
(16)	3.3	2.5	8	K	(16)	3.3	8.5	11	D

* Ovariectomized.

mental animals died before their controls and these were 3.3 and 4 months of age, respectively, at the beginning of the experiment. It is plain, therefore, that estrogen uniformly retarded the tuberculosis of animals beyond the age of sexual maturity. Before that age the effect of estrogen was not as constant. The possible significance of this observation will be discussed in the last paper of this series.

THE EFFECT OF CHORIONIC GONADOTROPIN, PROGESTERONE AND OVARIECTOMY ON TUBERCULOSIS

Since chorionic gonadotropin increases the spread of particles in the skin (4) and thus exercises an effect opposite to that of estrogen, it is obvious, if spread

is a factor in resistance to tuberculosis, that the appropriate administration of this hormone should enhance the tuberculous process.

Materials and Methods

Accordingly, the following experiment on female rabbits was set up. Litter mates, or rabbits descended from at least one common parent of the seventh to the ninth inbred generations of the resistant A family, were divided into two groups. One group served as control; the others were given 0.02 to 0.2 mg. chorionic gonadotropin (6) intravenously. About one week later, when about 10 actively functioning corpora lutea had developed in the ovaries of similarly treated rabbits, both groups were injected intracutaneously with the same suspension of virulent bovine tubercle bacilli of the Ravenel strain. The control animals received no treatment. The experimental animals continued to receive the gonadotropin intravenously every tenth day throughout the course of the disease, in order to induce fresh crops of corpora lutea at a time when the preceding secretory bodies were beginning to undergo regression.[5] The progress of the lesion at the site of inoculation was carefully measured throughout the course of the infection. Some pairs were allowed to die from their disease in order to determine the duration of the disease in the two groups. In other instances, if one of a pair died its mate was killed on the same day. The extent of the disease in the various organs was carefully ascertained both macroscopically and microscopically.

In text figure 6 may be seen the average progress of the lesion at the site of inoculation throughout the course of the disease in 4 rabbits under the influence of gonadotropin as compared with that in 4 untreated litter mates. It is clear that the volume of the lesion in the rabbits under the influence of the gonadotropin is consistently larger than that of the control rabbits.

In table 6 are listed all the pertinent data of the above 4 pairs as well as those of additional control and gonadotropin-treated animals studied on another occasion. For the sake of brevity they are placed together in one table. All the rabbits were sexually mature and thus were capable of fully responding to the gonadotropin. The close genetic relationship, if not identity, of these two groups is shown in column 3. That the progress of the lesion at the site of inoculation was uniformly enhanced in the gonadotropin-treated animals is shown in column 4 and illustrated in figures 3 and 4 (Plate I). In the majority of instances the extent of the disease in the internal organs was definitely enhanced and in 6 of the 7 pairs of experimental animals the degree of dissemination of the disease by hematogenous and lymphogenous routes was definitely greater than in the controls. It is noteworthy that there was a definite tendency for a shorter duration of the disease in the experimental animals. That the gonadotropin-treated animals were under the physiologic influence of the hormone is shown in the last column of this table, where it may be seen that all the gonadotropin animals showed either corpora lutea in the ovary or diffuse luteinization of its stroma.

That these results are specific for gonadotropin and that the observed differ-

[5] We are indebted to Dr. Gregory Pincus who suggested this procedure and to Dr. Samuel Gurin who furnished the highly purified gonadotropin.

ences could not be attributed to the varying natural resistance of these rabbits was shown by the following observations. Four litter mates of the first 4 pairs listed in table 6 were given daily subcutaneous injections of 1 to 2 mg. of progesterone together with 5 to 10 γ of estradiol (7) for two weeks before infection and every day thereafter throughout the course of the disease. It was found that this treatment exerted no discernible effect either on the progression of the lesion at the site of inoculation, the extent of the disease in the internal organs, or the dissemination of the tuberculosis by vascular channels as compared with

TABLE 6

The effect of chorionic gonadtropin on the course of tuberculosis in female rabbits of the highly inbred A family

GROUP	RABBIT NUMBER AND AGE IN MONTHS	PARENTS	VOLUME OF LOCAL LESION SIX WEEKS AFTER INFECTION	EXTENT OF PULMONARY LESIONS	EXTENT OF PLEURAL LESIONS	EXTENT OF RENAL LESIONS	SURVIVAL	NUMBER OF ORGANS METASTASIZED	PRESENCE OF CORPORA LUTEA OR LUTINIZED STROMA
							days		
Treated with gonadotropin	A9=56, 8	A8=61 x A8=62	433	+++±	++	++±	130	14	+
	A9=57, 8.5	A8=61 x A8=62	369	++±	++++	++±	149	7	+
	A9=43, 10	A8=66 x A8=62	340	++	+	+	K	5	+
	A9=63, 7.8	A8=59 x A8=64	299	++	++	++	K	12	Corpora hemorrhagica
	A8=20, 13	A7=15 x A7=1	528	+++++	++±	++±	158	6	+
	A7=24, 23	A6=6 x A6=18	1,233	+++	++++	++±	84	8	+
	A7=39, 11.5	A6=22 x A6=27	416	++±	++++	++	99	8	+
	Average....		517	3	2.8	2.1	126	8.6	
Control	A9=54, 8	A8=61 x A8=62	85	+	±	±	K	6	0
	A9=57, 10	A8=61 x A8=62	306	+	+	±	K	3	0
	A9=40, 10	A8=66 x A8=62	176	+++	++	++	172	6	0
	A9=44, 10	A8=66 x A8=62	213	0	±	0	K	1	0
	A9=61, 7.8	A8=59 x A8=64	180	++++	++	++	133	5	0
	A8=38, 10	A7=15 x A7=1	475	+	++	0	204	4	0
	A7=29, 17	A6=22 x A6=18	186	+++	+++	+	160	5	0
	A8=32, 10.3	A7=3 x A6=27	62	+++	+++	+++	95	4	0
	A8=22, 13	A7=13 x A7=1	338	++±	++±	++	134	9	0
	A8=45, 8.6	A7=13 x A7=25	62	+±	+±	±	204	4	0
	Average....		208	2	1.8	1.1	157	4.7	0

that in the controls listed in the table. Likewise, ovariectomy exercised no consistent effect on genetically closely related animals. As these two procedures exercised no effect on the tuberculous process, the data are not listed in detail.

Discussion

It has been demonstrated above that in mature, highly inbred litter mates the synthetic preparation of the naturally occurring estrogenic hormone, α estradiol dipropionate, uniformly retarded the tuberculous process at the site of inoculation in the skin, diminished the extent of the disease in the internal organs, and suppressed to a considerable degree its dissemination in the body.

In rabbits under the age of 4.8 months estrogen exercised a less constant effect and in some instances the tuberculous process was intensified in the internal organs.

By contrast, in a small series of highly inbred mature rabbits, the periodic administration of chorionic gonadotropin, which induced successive crops of corpora lutea in the ovary in the early phase of the disease, uniformly enhanced the tuberculous process at the site of cutaneous inoculation, increased its dissemination, and in the majority of instances aggravated the extent of the disease in the internal organs. Although the number of animals involved was small, the effect appeared specific, for ovariectomy or the daily administration of physiologic quantities of progesterone with several γ of γ estradiol to genetically similar animals exercised no effect on the process. The lack of complete uniformity in the influence of chorionic gonadotropin on the tuberculosis may be related to the inconstant development of fully formed corpora lutea in the ovary throughout the course of the disease in response to the repeated gonadotropic stimulations. As no rabbit gonadotropin has been isolated and, perforce, the human protein was used, it may not be surprising that occasionally the sera of the rabbits chronically treated with the gonadotropin inactivated the hormone, as had been observed by others (8).

Because of this inherent difficulty no further studies were attempted. Nevertheless, the results strongly suggest that gonadotropin affects the tuberculous process in a manner opposite to that of estrogen.

The observed effect of estrogen on the tuberculous process is in accord with the studies of Sprunt and McDearman (9), Foley and Aycock (10) and von Haam and Rosenfeld (11), who have shown that estrogen exerts a protective influence against acute infections caused by vaccinia virus, Streptococcus and Pneumococcus, respectively. However, investigators of the effect of estrogen in the chronic infection, tuberculosis, are quite contradictory in their conclusions. Some assert that it enhances the disease (12), others hold that it exerts no effect on the process (13). Similar differences of opinion have been expressed on the effect of gonadotropin on tuberculosis (14). These varying conclusions suggest that the effects cannot be of great magnitude. In the present experiments the factor of the natural resistance of the control and experimental animals was carefully balanced by the use of highly inbred litter mates of genetically uniform stock and of closely similar hereditary resistance to the disease (1). Under such conditions it should be possible to observe an effect which might be masked by natural variations in resistance of genetically differing animals. This was indeed the case. Furthermore, it has been shown by Sprunt and confirmed in the present studies that estrogen retards the spread of particles in the skin. For this and other reasons the cutaneous portal of entry was used. As it has been shown by Thomas and Duran-Reynals (15) that the incorporation of hyaluronidase in the oculum of tubercle bacilli injected intracutaneously enhances the disease, it is to be expected that a natural hormone which increases the connective tissue permeability to particulate matter would also accelerate the tuberculous process.

As will be shown in subsequent papers, however, mere reduction in spread is not the only effect exercised by estrogen on the rabbit and hence may not be the only effective mechanism by which it retards the disease.

To what extent these data apply to human tuberculosis is problematical. It is well known that the incidence of tuberculous infection as distinguished from clinically significant tuberculous disease increases regularly with age. However, the mortality and morbidity of the disease rises disproportionately synchronous with the onset of puberty, particularly in the female. This sudden change can be understood on the hypothesis that the sex hormones exercise the same effects in man and rabbits. With the onset of the menstrual cycle, a latent tuberculous focus is alternately under the influence of estrogen in the first portion of the cycle and under the corpus luteum, for some time preceding menstruation, in the latter half. It is conceivable that estrogen retards the spread of the disease in the first portion of the cycle, as it does in rabbits, whereas in the latter half the reduction of estrogen, together with the activity of the corpus luteum, induces a spurt of spread in the heretofore latent focus in the same manner as the corpora lutea induced by gonadotropin appear to increase the dissemination of the disease in the animal. However, as the estrogen used in these experiments far exceeds the physiological levels, it is questionable whether these observations can be directly applied to the human disease.

Summary

In highly inbred, sexually mature rabbits estrogen in large doses uniformly retarded the progress of the disease at the site of intracutaneous inoculation, diminished the extent of the disease in the internal organs, and suppressed to a considerable degree its dissemination in the body. In immature rabbits the estrogen was less effective on the spread of the disease. In a smaller group of highly inbred rabbits the periodic administration of chorionic gonadotropin, which induced corpora lutea in the ovary in the earlier phase of the disease, uniformly enhanced the progress of the disease at the site of intracutaneous inoculation, increased its dissemination in the body, and in the majority of instances aggravated its extent in the internal organs. Ovariectomy or the daily combined injection of physiologic amounts of progesterone and estradiol exercised no effect. The possible significance of these hormone effects in the pathogenesis of puberty tuberculosis in the human female is discussed.

Sumario

Factores Constitucionales en la Resistencia e la Infección: I Efecto del Estrógeno y de la Gonadotropina Coriónica sobre la Evolución de la Tuberculosis

En los conejos sexualmente maduros y muy entrecruzados, el estrógeno a grandes dosis retardó el proceso tuberculoso en el sitio de la inoculación intracutánea, disminuyó la extensión de la enfermedad en los órganos internos y suprimió considerablemente su difusión en el cuerpo. En los conejos inmaduros el estrógeno mostró menos efecto sobre la difusión. En un grupo más pequeño de conejos muy entrecruzados, la administración periódica de gonado-

tropina coriónica, que provocó la aparición de cuerpos amarillos en el ovario en la fase más temprana de la dolencia, intensificó invariablemente la agravación de la enfermedad en el sitio de la inoculación intracutánea, acrecentó la difusión en el cuerpo y, en la mayoría de los casos, agravó su extensión en los órganos internos. Ni la ovariectomía ni la inyección combinada a diario de cantidades fisiológicas de progesterona y estradiol ejercieron efecto alguno. Discútese la posible importancia de estos efectos hormónicos sobre la patogenia de la tuberculosis púber en la hembra humana.

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CONSTITUTIONAL FACTORS IN RESISTANCE TO INFECTION[1, 2]

II. The Effect of Estrogen on Tuberculin Skin Sensitivity and on the Allergy of the Internal Tissues

MAX B. LURIE, T. N. HARRIS, SAMUEL ABRAMSON[3] AND J. MARVIN ALLISON

Introduction

It was demonstrated in the preceding paper (1) that in mature highly inbred rabbits of genetically similar resistance to tuberculosis, estrogen uniformly retarded the progress of the disease at the site of intracutaneous inoculation and diminished its extent and dissemination in the internal organs. These observations may be understood in the light of the work of Sprunt and his collaborators (2) who found that estrogen reduced the spread of particulate matter in the skin and enhanced the resistance of rabbits to vaccinia virus. Conversely, Thomas and Duran-Reynals (3) have shown that by increasing the penetration of the infecting microorganisms at the portal of entry by the use of a testicular spreading factor, presumably hyaluronidase, the tuberculous process is enhanced not only at the site of inoculation but also in the entire body. Similarly it was found in the preceding study (1) that the periodic injection of chorionic gonadotropin, which engenders successive crops of corpora lutea in the ovary, increases the connective tissue permeability to particles and likewise tends to accelerate the progress of the disease.

While there is a certain degree of parallelism in the present studies between variations in tissue permeability and corresponding differences in the restriction of the disease in the connective tissues, this is far from complete. Hence, further studies were undertaken to elucidate other mechanisms by which estrogen might increase resistance to tuberculosis. As allergic sensitivity is one of the central phenomena in this disease, studies were undertaken to determine the effect of estrogen on the sensitivity of the skin to tuberculin and on the concomitant allergy of the internal organs and tissues.

Materials and Methods

The various groups of estrogen-treated and control rabbits infected with virulent tubercle bacilli, which were described in the previous paper (1), were injected intracutaneously with 0.1 cc. of a 1:10 dilution of the same Old Tuberculin preparation at weekly intervals throughout the course of their disease. The volume of inflammation that developed two days after the injection was determined according to a formula previously given (4) and served as a measure of the degree of skin sensitivity. Similar tests were made on tuberculous rabbits under the respective influence of chorionic gonadotropin, physiologic amounts of progesterone and estradiol, and ovariectomy.

[1] From The Henry Phipps Institute and the Children's Hospital, Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Aided by grants from the Commonwealth Fund and the Life Insurance Medical Research Fund.

[3] Tuberculosis Control Division, Public Health Service, Federal Security Agency.

Estrogen-treated rabbits and controls were also sensitized by the intracutaneous injection of identical amounts of heat-killed bovine tubercle bacilli at the same intervals. This was done to determine the effect of this hormone on skin sensitivity in the absence of active disease. As heretofore, 0.5 mg. of α-estradiol dipropionate in 0.5 cc. of sesame oil was given to the experimental rabbits at weekly intervals for fourteen days before the beginning of sensitization and throughout the study thereafter. The control animals received 0.5 cc. of sesame oil without the hormone at the same time.

Since the tuberculin sensitivity induced by treatment with heat-killed tubercle bacilli lasts for many months, and, furthermore, since injected estrogen is eliminated from the body, the effect on skin allergy of withdrawing the hormone and subsequently readministering it was studied.

To determine whether estrogen has the same effect on the sensitivity of the skin and on that of the internal tissues, control and estrogen-treated rabbits, sensitized with heat-killed tubercle bacilli, were injected with 0.2 cc. of a 1:10 dilution of tuberculin subcutaneously instead of intracutaneously. The response was measured by the rise in the absolute number of circulating polymorphonuclear leucocytes. For the same purpose, the extent of caseation in the tuberculous tissues of the estrogen-treated and control animals at death was correlated with the tuberculin skin sensitivity during the animal's life. Tissue culture studies on the effect of tuberculin on white blood cells derived from estrogen-treated and control rabbits, all sensitized with heat-killed tubercle bacilli, were also performed with the same object in view. Methods used in these experiments will be described in detail later. Finally, it became apparent during the course of this work that estrogen might affect the inflammation which a particular agent induced in the tissues apart from the phenomena of sensitization. Hence, several toxic substances were injected into the skin of unsensitized estrogen-treated and control animals, and the volume of the ensuing inflammation was measured as heretofore.

Results

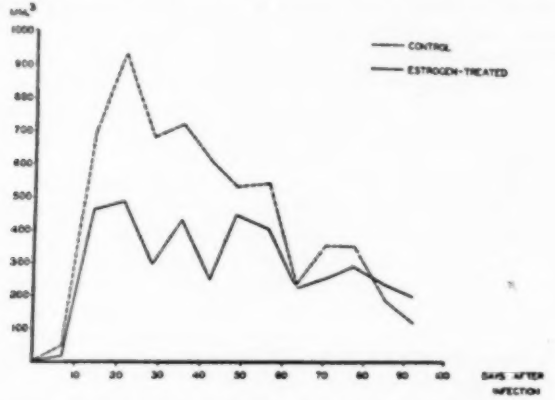
The effect of estrogen, chorionic gonadotropin, progesterone and ovariectomy on the tuberculin skin sensitivity of rabbits with progressive tuberculosis

Text figure 1 presents the average skin sensitivity in 4 estrogen-treated and 4 litter mate controls of the C family throughout the course of their disease. All the relevant data for this experiment of 1945–1946 are detailed in text figure 3 and table 2 of the preceding paper (1). It is evident that estrogen markedly and consistently reduced the skin sensitivity to tuberculin until very late in the course of the infection, at which time the less extensively diseased hormone-treated animals showed a somewhat higher sensitivity than their controls who were on the verge of death. As is well known, tuberculin skin sensitivity declines markedly during the last stages of tuberculosis.

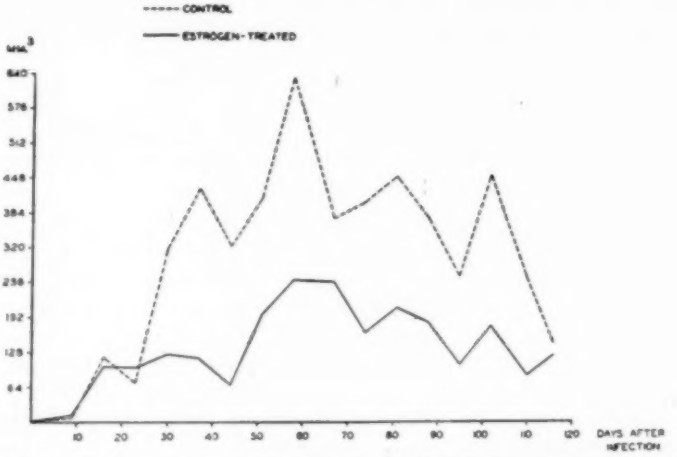
Text figures 2 and 3 present similar observations in the experiments of 1946–1947 on rabbits of the C and A families, respectively, which are detailed in text figures 4 and 5 and tables 3 and 4 of the preceding study (1). It is again clear that estrogen uniformly reduced the skin sensitivity in the same manner as in the experiment of 1945–1946.

Text figure 4 shows the average degree of tuberculin sensitivity of the skin at different intervals during the course of the disease in 5 control rabbits of the

Family A, and in 8 litter mates, 4 of which were under the influence of chorionic gonadotropin and 4 under daily treatment with physiologic quantities of progesterone and several micrograms of estradiol. The exact conditions under which

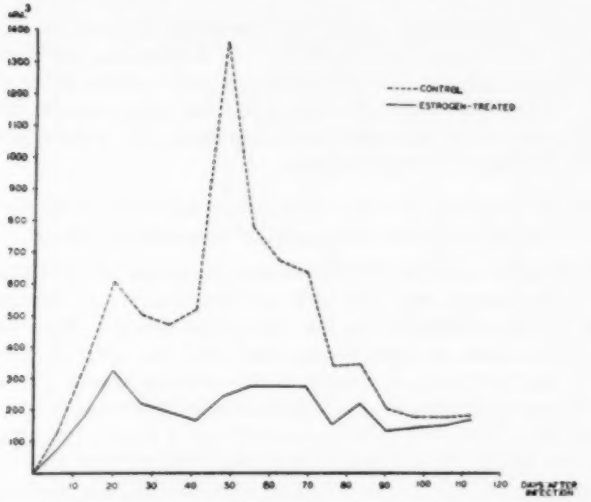


TEXT FIGURE 1. Average volume of tuberculin reaction during course of infection in estrogen-treated and control litter mates of the C family (Experiment of 1945–1946).

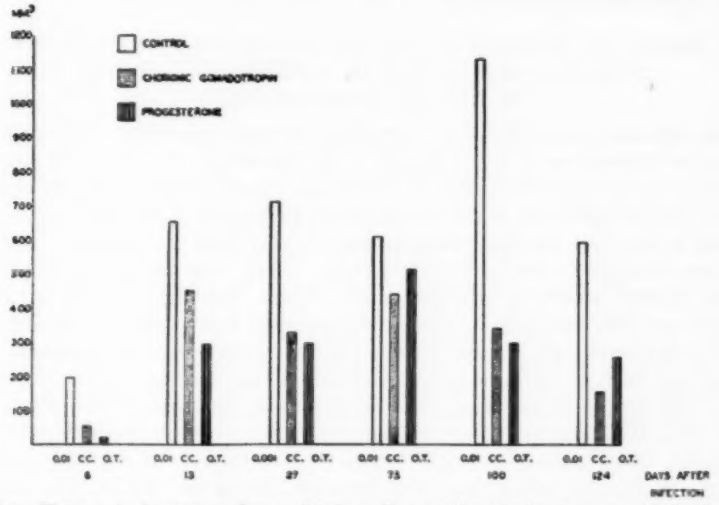


TEXT FIGURE 2. Average volume of tuberculin reaction during course of infection in estrogen-treated and control litter mates of the C family (Experiment of 1946–1947).

these data were obtained from the control and gonadotropin rabbits are given in text figure 6 and table 6 of the preceding report. It is evident that treatment of rabbits with chorionic gonadotropin, which it will be remembered (1) tended to enhance the disease, and progesterone-estrogen injections, which did not ma-



TEXT FIGURE 3. Average volume of tuberculin reaction during course of infection in estrogen-treated and control litter mates of the A family (Experiment of 1946–1947).



TEXT FIGURE 4. Average volume of tuberculin reaction during course of infection in chorionic gonadotropin-treated, progesterone-treated and control litter mates of the A family (Experiment of 1944–1945).

terially affect the process, both consistently lowered the skin sensitivity as compared with that observed in their litter mate controls. Furthermore, ovar-

iectomy, which was found to exercise no discernible influence on the progress of the disease, also lowered skin sensitivity. It is apparent, therefore, that the degree of tuberculin skin sensitivity has no constant relation to the progress of the disease. Hence, the observed retardation of the tuberculosis in the estrogen-treated litter mates is not accounted for by the depression of the skin sensitivity which follows treatment by this hormone.

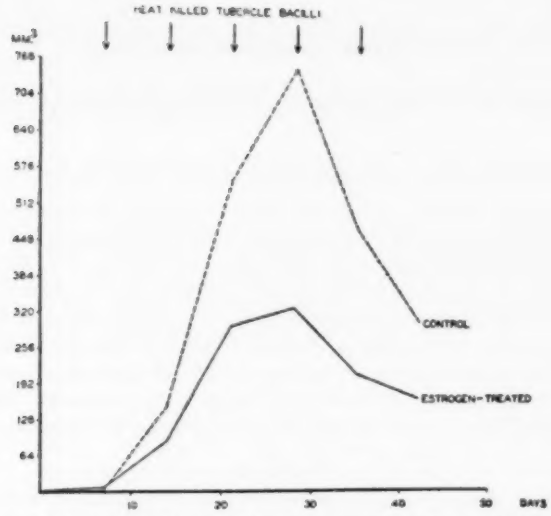
THE EFFECT OF ESTROGEN ON THE TUBERCULIN SKIN SENSITIVITY OF RABBITS SENSITIZED WITH HEAT-KILLED TUBERCLE BACILLI

While there is no strict parallelism between the extent of the disease and the intensity of skin allergy, one may still contend that, *ceteris paribus*, the less marked the antigenic stimulus the less will be the allergy. Since estrogen retarded the tuberculosis, it might be presumed that one factor in this reduction of allergy is the less extensive disease of the estrogen-treated animals. That this is not the case is illustrated in text figure 5 which presents the average degree of tuberculin sensitivity in 5 estrogen-treated and 5 control genetically closely related rabbits on each of six successive weeks. All of these rabbits had been given the same quantities of heat-killed tubercle bacilli intracutaneously at the same intervals as indicated in the figure. It is evident, therefore, that identical nonmultiplying antigenic stimuli result in a lower skin sensitivity in estrogen-treated than in control animals.

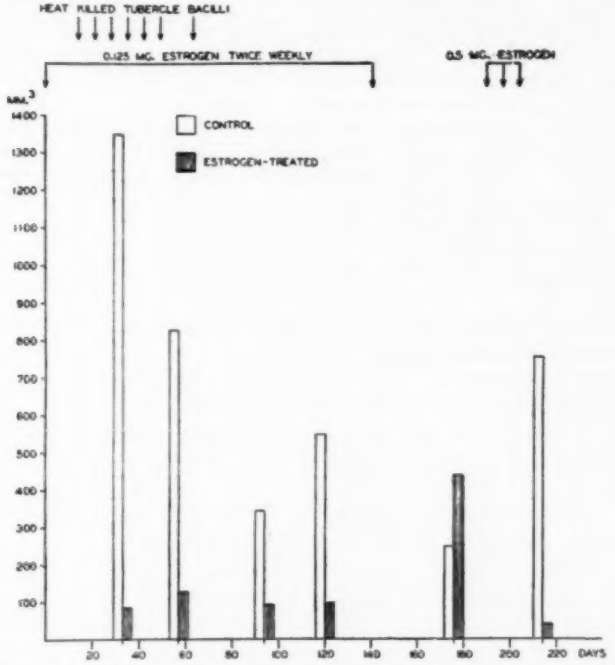
THE EFFECT OF WITHDRAWAL AND READMINISTRATION OF ESTROGEN ON TUBERCULIN SKIN ALLERGY IN RABBITS SENSITIZED WITH HEAT-KILLED TUBERCLE BACILLI

The depressed skin allergy to tuberculin in estrogen-treated rabbits sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli may be due to a fundamental depression of the sensitizing mechanism or it may simply result from an alteration of the inflammatory irritability of the skin. That the second is the more likely explanation may be seen from text figure 6.

Three estrogen-treated and three control rabbits were sensitized with heat-killed tubercle bacilli as indicated in the figure. For the first 140 days the rabbits received 0.125 mg. α-estradiol dipropionate twice weekly. During this time both groups were repeatedly tested with tuberclin. In each instance the sensitivity of the rabbits under the influence of the hormone was consistently lower than that of the controls. The hormone was now withdrawn and thirty-six days after the last injection of estrogen the rabbits were again tested with tuberculin. As is evident from the figure, the sensitivity was reversed and the rabbits, whose skin allergy had been depressed during the estrogen administration, now reacted with even greater intensity than the control animals. The experimental rabbits were then again given estrogen for three weeks. The sensitivity of the estrogen-treated rabbits was again markedly depressed. Thirty-six days after the second hormone withdrawal, the sensitivity was again restored. It is clear, therefore, that the lowered sensitivity of the skin in rabbits under the influence of estrogen is not due to a depression of the level of skin sensitization,



TEXT FIGURE 5. Average volume of tuberculin reaction after sensitization with heat-killed tubercle bacilli in estrogen-treated and control inbred rabbits (Experiment of 1946).



TEXT FIGURE 6. The effect of estrogen and its withdrawal and readministration on tuberculin skin allergy in rabbits sensitized with heat-killed tubercle bacilli (Experiment of 1946–1947).

but to some effect of the hormone on the inflammatory response of the skin to tuberculin.

THE EFFECT OF ESTROGEN ON THE ALLERGIC SENSITIVITY OF THE INTERNAL TISSUES

The question now arose whether the body as a whole shares with the skin in the depression of the inflammatory responsiveness to the products of the tubercle bacillus as a result of estrogen administration. It was found, in the first place, that the extent of caseation in the tuberculous foci was in general at least as pronounced in the estrogen-treated animals as in their litter mate controls. Furthermore, in the former group, exudation into the alveoli of the lungs with necrosis was prominent in the advancing portion of the lesion, whereas in the latter animals, thickening of the alveolar septa by a productive inflammation was most marked and caseation was less widespread. If caseation is mediated

TABLE 1

The effect of estrogen on the rise of polymorphonuclear counts in tuberculin-sensitive rabbits induced by the subcutaneous injection of tuberculin

GROUP	RABBIT NUMBER	ORIGINAL NUMBER OF POLYMORPHONUCLEARS	NUMBER OF POLYMORPHONUCLEARS 24 HOURS AFTER TUBERCULIN	RISE IN POLYMORPHONUCLEARS	GROUP	RABBIT NUMBER	ORIGINAL NUMBER OF POLYMORPHONUCLEARS	NUMBER OF POLYMORPHONUCLEARS 24 HOURS AFTER TUBERCULIN	RISE IN POLYMORPHONUCLEARS
				per cent					*per cent*
Untreated controls	V 414	5,119	7,330	43.2	Estrogen-treated	V 267	2,936	7,712	162.6
	V 366	2,983	6,763	126.7		V 30	1,785	5,418	203.5
	A 6	1,760	7,893	348.4		A 5	1,625	4,956	204.8
	A 9	1,534	6,767	341.1		A 8	1,468	5,763	292.5
	A 11	2,303	5,486	138.2					
	Average...	2,740	6,848	199.5		Average...	1,953	5,962	215.8

by allergic sensitivity, for which there is some evidence, then estrogen does not reduce this sensitivity in the lungs.

It is difficult, however, to evaluate the extent of caseation in terms of allergic sensitivity if the complexity of the factors involved are considered. Therefore, in an effort to bypass the skin, control and estrogen-treated rabbits which had been sensitized with heat-killed tubercle bacilli were given a subcutaneous injection of 0.2 cc. of 1:10 dilution in saline of Old Tuberculin on Long's synthetic medium. The response was measured by the absolute rise in circulating polymorphonuclear leukocytes on the day following injection.

Table 1 shows the results of such an experiment. It is evident that sensitized rabbits, whether under the influence of estrogen or not, reacted to the subcutaneous injection of tuberculin with a sharp rise in polymorphonuclear leukocytes which is of the same degree in both groups. It is noteworthy that sterile Long's synthetic medium concentrated on the waterbath to one-tenth its original volume, *i.e.*, control material, caused no significant change in the leukocyte count

on subcutaneous injection. It is thus apparent that estrogen did not lower the leukocytic response of allergic rabbits to tuberculin.

THE TUBERCULIN SENSITIVITY OF EXPLANTS OF BLOOD LEUKOCYTES DERIVED FROM SENSITIZED, ESTROGEN-TREATED AND CONTROL RABBITS

Since the original tissue culture studies of Rich and Lewis (5) on the tuberculin reaction have shed the clearest light on the phenomenon, an attempt was made to determine whether any difference in cell sensitivity to tuberculin could be demonstrated in explants of blood leukocytes derived from estrogen-treated and control rabbits, sensitized with heat-killed tubercle bacilli.

Three estrogen-treated and three control rabbits were sensitized with heat-killed tubercle bacilli as detailed above in text figure 6. After repeated tuberculin skin tests had shown that in the rabbits under the influence of the hormone the inflammatory response was markedly depressed, blood was withdrawn from the heart of the rabbits of both groups. The citrated blood was centrifuged and the leucocytic layer was coagulated with 0.5 per cent calcium chloride in Gey's solution (6). The tissue was minced into pieces of approximately 1.0 mm. in diameter, washed with Gey's solution, and picked up by a Pasteur pipet bent at the tip and placed in a test tube 150 by 18 mm. The arrangement of the explants was as follows. Five bits of tissue from an experimental animal were placed in a straight line extending approximately halfway up the tube. A few drops of the clotting mixture were then run down the tube along the line of explants and allowed to remain in a horizontal position until coagulation had occurred. The same procedure was then repeated with five bits of tissue from a control animal along the opposite side of the same tube. The clotting mixture consisted of equal amounts of heparinized chicken plasma, and 10 day chick embryo extract in Gey's solution. The latter contained the appropriate dilution of Old Tuberculin prepared on Long's synthetic medium, or the concentrated sterile medium which served as control for the tuberculin. In the case of each row of explants the tube was manipulated so that each explant was surrounded by several millimeters of clot. The tubes were then stoppered and incubated at 37°C in a roller-tube mechanism. The cultures were examined after twenty-four hours and the farthest distance of migration of leucocytes from the periphery of the explant was measured by an ocular micrometer. These radii were determined in four directions at 90 degree intervals around the explant, and the mean was taken as the average distance of migration. The results are given in arbitrary units of the ocular micrometer used in these experiments.

In preliminary experiments it was found that the preparation of tuberculin used in these experiments showed a very marked inhibition of migration of the leukocytes of sensitized rabbits at a concentration of 1:500 and no statistically significant effect at 1:4,000. In order to allow an opportunity for the demonstration of any difference in sensitivity between estrogen-treated and control rabbits it was thought best to use the intermediate concentration of 1:1,000.

It was observed that a 1:1,000 solution of uninoculated medium showed no effect on the tissue cultures. Hence it was considered justifiable to attribute the inhibitory effect of tuberculin at concentrations of 1:1,000 to specific sensitization and to use saline solution as control material for the tuberculin.

In the major series of tissue culture experiments, ten explants of leukocytes from each rabbit were cultivated in the presence of tuberculin at concentrations

of 1:1,000, 1:4,000, and zero. The mean radius of migration of leucocytes for each of the 10 specimens is shown in table 2.

It will be seen that in all explants derived from both estrogen-treated and control animals there was a very small difference between cultures grown in saline and those grown in 1:4,000 tuberculin. In no instance, however, was the difference statistically significant. In contrast, the cultures grown in a medium containing tuberculin in a concentration of 1:1,000 all showed a marked decrease in radius of migration as compared with the control cultures. Analysis of the data by the Student method revealed this difference to be statistically significant.

A comparison of the effect of this concentration of tuberculin (1:1,000) on the migration of leukocytes derived from estrogen-treated and control animals showed that explants from two of the estrogen-treated rabbits were inhibited

TABLE 2

Average radius of migration in tissue culture of leukocytes derived from sensitized estrogen-treated and control rabbits in the presence of various concentrations of tuberculin together with the corresponding sensitivity of the skin to tuberculin†*

TREATED WITH ESTROGEN						CONTROL, UNTREATED					
Rabbit Number	Radius of migration in salt solution	Radius of migration in 1:4,000 tuberculin	Radius of migration in 1:1,000 tuberculin	Reduction of migration in 1:1,000 tuberculin	Skin sensitivity of inflammation	Rabbit Number	Radius of migration in salt solution	Radius of migration in 1:4,000 tuberculin	Radius of migration in 1:1,000 tuberculin	Reduction of migration in 1:1,000 tuberculin	Skin sensitivity of inflammation
				per cent	mm.**					*per cent*	mm.**
A 5	341	319	218	36.1	84	A 6	309	321	230	25.6	637
A 8	385	350	203	47.4	153	A 9	299	323	216	27.8	323
A 10	320	291	201	37.2	45	A 11	268	242	161	39.9	383

* Each value is an average of 10 explants expressed in arbitrary micrometer units.

† Represents the average value of inflammation to 0.1 cc. of 1:10 dilution of tuberculin two weeks before and four days after the leukocytes were cultured.

** Per cubic millimeter of free flowing, venous blood.

in their migration by tuberculin to a somewhat greater extent than those derived from two untreated sensitized controls. The explants from the remaining experimental and control rabbits were equally inhibited by this concentration of tuberculin. These differences, however, are not statistically significant.

By contrast, the skin sensitivity to tuberculin of each rabbit under influence of estrogen, tested two weeks before and four days after the tissue cultures were done, was markedly depressed in comparison with that of the sensitized controls.

It is evident, therefore, that tissue culture also failed to show any difference in the degree of cell sensitivity to tuberculin as a result of estrogen treatment.

THE INFLAMMATORY RESPONSE OF UNSENSITIZED ESTROGEN-TREATED AND CONTROL RABBITS TO BACTERIAL IRRITANTS

It is apparent from the work thus far reported that there is no evidence that estrogen depresses the allergic irritability of the internal tissues of sensitized

rabbits. Even what appears to be a reduction in the tuberculin sensitivity of the skin is not due to a lower degree of sensitization of this tissue, but rather to an alteration induced by the hormone in the inflammatory responsiveness of the skin to the irritant. That the intrinsic allergic irritability was unaffected was demonstrated by the withdrawal of the hormone.

TABLE 3

The effect of estrogen on the inflammatory irritability of the skin to heat-killed tubercle bacilli and to pertussis endotoxin

RESPONSE TO HEAT-KILLED TUBERCLE BACILLI			RESPONSE TO PERTUSSIS ENDOTOXIN		
Group	Rabbit number	Volume of inflammation	Group	Rabbit number	Volume of inflammation
		mm.³*			mm.³*
Treated with estrogen	V 30	44	Treated with estrogen	V 30	76
	V 366	34		V 366	81
	V 784	54		V 784	144
	V 267	26		V 267	28
	V 414	39		V 414	50
	Average.....	39		Average.....	76
Untreated controls	T 33	69	Untreated controls	T 33	68
	V 685	140		V 685	392
	V 368	85		V 368	432
	U 57	76		U 57	118
	S 376	97		S 376	54
	Average.....	93		Average.....	213

* Per cubic millimeter of free flowing, venous blood.

That this is the correct explanation for the reduction of the skin sensitivity by estrogen may be seen from the following observations.

Ten unsensitized genetically closely related rabbits were divided into two groups. Five were treated with estrogen as usual. The remaining animals served as controls. After the experimental animals had been under the influence of estrogen for twenty days, both groups received 1.0 mg. of heat-killed tubercle bacilli of the Ravenel strain intracutaneously. The extent of the inflammation at the site of injection was measured in both groups forty-eight hours later. On another occasion both groups were injected intracutaneously with 0.1 cc. of 1:200 dilution in saline of a sonic vibration extract of *Bacillus pertussis*[4] (7). On the second day the ensuing inflammation was measured. The results are presented in table 3.

It is evident that the inflammatory reaction to both bacterial agents is reduced in the skin of estrogen-treated rabbits. As the rabbits received these irritants for the first time, no phenomena of sensitization were involved. Thus two substances of primary toxicity to the skin induced less inflammation in animals under

[4] The pertussis endotoxin was kindly furnished by Dr. Joseph Smolens.

the influence of the hormone than in control rabbits. Therefore, the depression of the tuberculin skin reaction in sensitized rabbits treated with estrogen is due to a lower inflammatory responsiveness of the skin to bacterial irritants in general.

A similar depression of the inflammatory responsiveness of the skin was noted in a study of the Arthus phenomenon in estrogen-treated and control rabbits sensitized with a protein of the tubercle bacillus kindly furnished by Dr. F. B. Seibert (8). After sensitization to the protein had been achieved, and in the presence of approximately equal precipitin titres in the serum of both groups, the inflammation at the site of intracutaneous injection of the antigen was usually much less intense in the estrogen-treated animals.

Discussion

The inflammatory response to tuberculin of the skin of rabbits sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli is markedly depressed by the administration of estrogen. At the same time, however, the polymorphonuclear leukocytosis which follows the subcutaneous administration of tuberculin in sensitized rabbits is equally intense whether the rabbits are under the influence of the hormone or not. Similarly, explants of tissue derived from sensitized rabbits, whether estrogen-treated or not, are equally inhibited in their migration in tissue cultures in the presence of the same concentration of tuberculin. Furthermore, the extent of caseation in estrogen-treated rabbits with progressive tuberculosis is not only not reduced but may even be increased. Thus the internal tissues are not depressed in their allergic response to the tubercle bacillus or its products, as is the skin. Even what appears as a reduction of the skin sensitivity to tuberculin is not due to a depression of the acquired degree of sensitivity of the skin, as a result of an interference by estrogen with the sensitizing mechanism. For, on withdrawal of the hormone, the sensitivity of the skin, without any additional sensitization, becomes at least as pronounced as that of control rabbits similarly sensitized. This heightened sensitivity can now again be depressed by further estrogen administration. Estrogen thus masks an existing unaltered allergic irritability. Finally the skin of estrogen-treated rabbits responds with less inflammation to unrelated primary toxic agents given to unsensitized rabbits. Hence the reduction in the skin sensitivity to tuberculin of estrogen-treated rabbits, whether sensitized by heat-killed tubercle bacilli or by active disease, is due not to diminution of skin sensitization, but simply to a depression of the inflammatory responsiveness of the skin to toxic agents in general. Among these agents is tuberculin, which is toxic to sensitized rabbits.

Analysis of the effect of estrogen on the tuberculin sensitivity of the skin has failed to shed any light on the mechanism whereby this hormone retards the tuberculous process. Nevertheless, the studies reveal that under certain conditions the tuberculin reaction of the skin is an unreliable index not only of the degree of sensitization of the internal organs, but even of that of the skin itself. The phenomenon was observed with estrogen which masks an existing allergic irritability by depressing the inflammatory response of the skin to irritants in

general. The importance of this observation in the interpretation of the problem of the relation between allergy and immunity is obvious.

Summary and Conclusions

1. Estrogen reduced the inflammatory response of the skin to tuberculin in rabbits sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli by virtue of the depressing effect of the hormone on the inflammatory irritability of the skin to bacterial irritants in general.

2. The acquisition of intrinsic allergic sensitivity of the tissues in general, as well as that of the skin, is not reduced by estrogen administration.

Sumario

Factores Constitucionales en la Resistencia a la Infección: II. El Efecto del Estrógeno sobre la Cutisensibilidad a la Tuberculina y sobre la Alergia de los Tejidos Internos

1. El estrógeno atenuó la respuesta inflamatoria de la piel a la tuberculina en los conejos sensibilizados por tuberculosis activa o por tratamiento con bacilos tuberculosos muertos al calor, por virtud del efecto depresor que ejerce la hormona sobre la irritabilidad inflamatoria de la piel a los irritantes bacterianos en general.

2. La adquisición de sensibilidad alérgica intrínseca por los tejidos en general, así como por la piel, no es rebajada por la administración de estrógeno.

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CONSTITUTIONAL FACTORS IN RESISTANCE TO INFECTION[1,2]

III. On the Mode of Action of Estrogen and Gonadotropin on the Progress of Tuberculosis

MAX B. LURIE, SAMUEL ABRAMSON,[3] A. G. HEPPLESTON[4] AND MARVIN J. ALLISON

INTRODUCTION

In the preceding studies (1, 2) an endeavor was made to elucidate the mechanism whereby, on the one hand, estrogen retarded the progress of tuberculosis and, on the other, chorionic gonadotropin enhanced the dissemination of the disease. It was found (2) that estrogen markedly suppresses the inflammatory response of the skin to tuberculin in rabbits sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli. It was also found, however, that estrogen reduces the inflammatory response of the skin to bacterial toxic agents in general and hence also to tuberculin, which is toxic to sensitized animals. Therefore, this seemingly depressing effect of estrogen on allergy, one of the important elements in the tuberculous process, is not due to an essential alteration in the allergizing mechanism but rather to the masking of an existing unaffected allergy by the hormone. What role, if any, this reduction in the inflammatory response of the skin plays in the retardation of tuberculosis by the hormone is not clear.

In a further effort to demarcate the means by which estrogen retards the progress of tuberculosis, information was sought as to the effect of estrogen on the rate of multiplication of tubercle bacilli at the site of inoculation in the skin and in the metastatic foci. Bacterial infection takes place chiefly in the connective tissue. As the outstanding effect of estrogen on tuberculosis is its restricting influence on the dissemination of the infection, whereas that of gonadotropin is the enhancement of this process, studies on the alterations produced by these hormones in the constituents of the connective tissue were instituted. An endeavor was made to determine whether estrogen affected the concentration of water in the connective tissue of the skin. Studies were also undertaken to determine the effect of estrogen and chorionic gonadotropin on the hyaluronic acid of the skin and on its vascular permeability; for these factors play a significant role in the dissemination of particles and bacteria in the connective tissues.

As stated in the second paper of this series (2), the parallel observed between the effect of estrogen on the spread of particles on the one hand, and on the progress of the disease on the other, was not constant. Therefore, other clues were sought to explain the observations.

[1] From The Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Aided by a grant from the Commonwealth Fund.

[3] Tuberculosis Control Division, Public Health Service, Federal Security Agency.

[4] Dorothy Temple Cross Research Fellow of the Medical Research Council of Great Britain.

In view of the well known interrelation between the gonads and the adrenals and the studies of Selye (3) on the adaptation syndrome and, in view of the demonstration by White and Dougherty (4) of the controlling effect of the adrenal corticosterones on the lymphocytes and their dissolution, and furthermore in consideration of the observations of McMaster and Hudack (5) and Ehrich and Harris (6), which strongly suggest that lymphocytes play a significant role in antibody production, the effect of the administration of estrogen on the adrenal cortex and on antibody formation was studied. The influence of this hormone on the thyroid gland was also noted.

Finally, during the course of this investigation, it was found that estrogen markedly suppressed the usually extensive amyloid degeneration in the spleen of rabbits with chronic tuberculosis. In the following pages these data are evaluated and an attempt is made to integrate the observations in the light of present knowledge.

TABLE 1

Fate of tubercle bacilli at the portal of entry and in the draining nodes and kidneys of estrogen-treated and normal rabbits

RABBIT NUMBER		DAYS AFTER INFECTION	NUMBER OF VIABLE TUBERCLE BACILLI ON CULTURE					
			Local lesion		Draining nodes		Kidney	
Experimental; age in months at time of infection	Control; age in months at time of infection		Experimental	Control	Experimental	Control	Experimental	Control
A9=98; 17	A9=99; 17	2	80	624	750	1,225	1	1
A9=102; 17	A9=103; 17	7	1,140	1,116	29,800	14,520	0	0
A9=135; 4	A9=138; 4	14	56,000	43,000	260,000	—	0	?
A10=7; 4	A10=10; 4	28	11,000	2,000	106,500	133,500	0	0

Observations

THE EFFECT OF ESTROGEN ON THE RATE OF MULTIPLICATION OF TUBERCLE BACILLI IN THE TISSUES

It was reported by Faulkner (7) that the estrogenic substance, diethylstilbestrol, is bactericidal to tubercle bacilli *in vitro*. It became necessary, therefore, to determine whether the natural estrogen, estradiol, used in these studies retarded the tuberculous process by suppressing the multiplication of the bacilli in the tissues. To answer this question, four litter mate pairs of the A family were divided into two groups. One received 0.5 mg. of α-estradiol dipropionate subcutaneously in sesame oil once a week, and the other received the same amount of oil without the hormone. When the experimental animals had been under the influence of estrogen for two weeks, both groups were infected intracutaneously with virulent bovine tubercle bacilli. At various intervals following infection, weighed amounts of the cutaneous lesion, the draining lymph nodes, and the internal organs were cultured on a modified Löwenstein medium (8) and the number of viable tubercle bacilli that they contained was determined as

described in previous studies (9). Estrogen was administered at weekly intervals throughout the course of the experiment.

The results are presented in table 1. It is evident that estrogen exercises no constant effect on the growth of tubercle bacilli at the site of inoculation or in the metastatic foci in the early stages of the infection.

THE EFFECT OF ESTROGEN AND GONADOTROPIN ON THE CONSTITUENTS OF THE CONNECTIVE TISSUE OF THE SKIN

Table 2 shows the effect of estrogen treatment on the spread of India ink in the rabbits of the experiment cited in the above paragraph, previous to infection. Included in this table are similar data on a group of litter mates of the C family. Clearly, estrogen usually restricts the spread of particles in the skin to a considerable degree, as shown previously by Sprunt (10).

TABLE 2

The effect of estrogen on the spread of India ink particles in the skin

GROUP	RABBIT NUMBER	1 DAY SPREAD MM²*	RABBIT NUMBER	1 DAY SPREAD MM²*
Untreated controls	A9=99	1,187	C8=2	1,470
	A9=103	1,800	C8=17	2,122
	A9=138	1,458	C8=19	1,850
	A10=10	1,722	C8=23	2,787
	Average.....	1,542	Average	2,057
Estrogen treated	A9=98	567	C8=1	1,120
	A9=102	1,367	C8=16	1,410
	A9=135	1,361	C8=18	1,990
	A10=7	967	C8=24	1,120
	Average.....	1,066	Average	1,410

* Area of spread in square millimeters as determined by planimeter.

THE EFFECT OF ESTROGEN ON THE CONCENTRATION OF WATER IN THE SKIN

As stated above, it is possible that much of the retardation of the progress of tuberculosis induced by estrogen may be due to its restricting effect on the spread of particles, such as tubercle bacilli, in the tissues. Therefore, the mechanism whereby estrogen reduces the permeability of the connective tissue was investigated.

It is well known that estrogen produces a great increase in the water content of the sexual skin of monkeys. Furthermore, it has been demonstrated by Zuckerman (11) that estrogen increases the water of the skin of mice. If rabbits are similarly affected by the hormone, a simple explanation for the restricting effect of estrogen on the spread of particles would be at hand. The water in the connective tissue is not in a free state but is either intracellular or bound to the matrix or in a film about the fibres and cells (12). As a result of the introduction of estrogen, these cells and fibres may swell. Thus the turgescence of the connec-

tive tissue elements would increase and in this way hinder their disruption by particles introduced into their interstices. Accordingly, the water content of the skin of estrogen-treated and control, litter mate rabbits was determined.

About one gram of clipped skin was excised with sharp, curved scissors. After removing the adhering blood with gauze, the skin was quickly placed in a previously accurately weighed covered bottle and heated to a constant weight at 100° C. From these data the concentration of water in the skin of estrogen-treated and control animals was determined. The observations are presented in table 3.

It is evident that estrogen does not increase the total concentration of the water in the skin. Whether the hormone alters the distribution of water between the extra- and intracellular compartments has not been determined.

TABLE 3

The effect of estrogen on the concentration of water in the skin

GROUP	RABBIT NUMBER	PER CENT OF WATER	GROUP	RABBIT NUMBER	PER CENT OF WATER
Untreated controls	T 33	67	Untreated controls	A9=99	63.3
	V 685	69		A9=103	67.9
	V 368	67		A9=138	71.3
	U 57	69		A10=10	75.4
	S 376	65.5			
	Average.....	67.5		Average.....	69.5
Treated with estrogen	V 30	65	Treated with estrogen	A9=98	69.0
	V 366	67		A9=102	68.4
	U 784	68		A9=135	74.2
	V 267	67		A10=7	68.5
	V 414	69.7			
	Average.....	67.3		Average.....	70.0

THE EFFECT OF ESTROGEN AND CHORIONIC GONADOTROPIN ON THE SPREADING INFLUENCE OF HYALURONIDASE

It was shown in a previous study (13) that chorionic gonadotropin enhances the spread of India ink in the skin. The process is mediated by the corpora lutea engendered in the ovary by the gonadotropin for in ovariectomized animals this hormone has no such effect. Furthermore, progesterone also tends to enhance the spread of dyes in the skin. Estrogen exercises an opposite effect, restricting the spread of particles. Hence it was thought possible in connection with the permeability of the skin, that the interrelation between the enzyme hyaluronidase and its substrate hyaluronic acid may be under the influence of the sex hormones (14). Further support for this notion is afforded by the fact that a substance similar to hyaluronic acid accumulates (15) in the sex skin of monkeys, under the influence of estrogen.

Accordingly 3 litter mates of the tenth inbred generation of the A family were divided into three classes. Five such litter mate groups were thus divided. The first class of 5 rabbits received 0.5 mg. α-estradiol dipropionate subcutaneously in 0.5 cc. sesame oil at weekly intervals for a total period of three weeks. The second class of 5 rabbits served as controls and received the same weekly injection of oil without the hormone. The last 5 rabbits received 0.06 mg. of chorionic gonadotropin intravenously at ten day intervals. This amount of gonadotropin regularly engendered crops of corpora lutea in the ovary.

Six days after the last injection of estrogen and two days after the last treatment with chorionic gonadotropin, the spreading effect of hyaluronidase[5] on India ink and on rabbit hemoglobin injected in the skin of the above three groups of litter mates was measured. It was believed that, if the character or concentration of hyaluronic acid in the skin of animals under the influence of these two hormones differs, the same amount of hyaluronidase would exercise a differential spreading effect in animals subjected to the respective hormone.

Three tenths cc. of saline containing the same amount of India ink or hemoglobin without hyaluronidase, and the same volume and concentration of the two dyes containing 1 and 5 γ of the enzyme per cc., respectively, were injected into corresponding areas of each of the 15 rabbits in the three above groups. The spread of these two dyes in the six sites of each of these rabbits was measured by calipers fifteen minutes after injection. The area of spread was determined as suggested by Hechter (16).

An examination of table 4, which summarizes these observations, reveals that under all conditions, with or without hyaluronidase, the spread of India ink particles or of diffusible rabbit hemoglobin tends to be restricted in the estrogen-treated rabbits and enhanced in rabbits under the influence of gonadotropin as compared with that in untreated litter mates. This confirms the original observations (13).

In general, 5 γ of hyaluronidase per cc. enhanced the spread of both dyes to a greater extent than 1 γ. Apparently, therefore, the enhancement of spread by hyaluronidase is proportional to its concentration. Furthermore, hyaluronidase has a much greater enhancing effect on the spread of diffusible hemoglobin than on that of particulate India ink. As tubercle bacilli are particulate, it is evident that, from the standpoint of the mechanism of action of these hormones on the tuberculous process, the effect of hyaluronidase on the spread of particulate ink in rabbits under the influence of these two hormones is of greater significance than the effect of this enzyme on diffusible hemoglobin.

If attention is concentrated on the effect of both doses of hyaluronidase on the spread of India ink in estrogen-treated rabbits and in litter mates under the influence of gonadotropin, it is evident that the enzyme enhances the spread of particles to a greater extent in the former than in the latter. This difference, though only 20 per cent in extent, is, nevertheless, statistically significant, for the critical ratio of the difference is 3.3 with a "P" value of 0.002. Essentially

[5] Highly purified sheep testicle hyaluronidase (VIII 128) was kindly placed at our disposal by Dr. Karl Meyer.

the same diminution in the spreading effect of hyaluronidase on hemoglobin was seen in animals under the influence of gonadotropin as distinguished from those under the effects of estrogen. In this case, however, the difference was not quite statistically significant for the "P" value was 0.04. Furthermore, hyaluronidase enhanced the spread of India ink to a slightly greater extent in estrogen-treated rabbits than in normal litter mates. The difference was observed in only half of the tests, however, and the results were not statistically significant.

The enhancement of spread induced by hyaluronidase is due to the hydrolysis of the hyaluronic acid matrix of the connective tissue (14). Therefore, the enzyme depolymerizes this intercellular substance over a wider area or more completely in estrogen-treated rabbits than in animals under the influence of gonadotropin. A similar though less constant difference would appear to obtain

TABLE 4

The effect of hyaluronidase on the spread of India ink in the skin of normal, estrogen-treated, and gonadotropin-treated litter mates of the inbred rabbit family A

GROUP	NUMBER OF RABBITS	SPREAD OF INDIA INK IN MM²					SPREAD OF HEMOGLOBIN IN MM²		
		Without hyaluronidase	With hyaluronidase		Average of 10 observations	Increment of spread for both doses	Without hyaluronidase	With 5 γ of hyaluronidase per cc.	Increment of spread
			1 γ/cc.	5 γ/cc.					
Normal	5	221	233	283	259	1.2 ± 0.06	285	595	2.1 ± 0.11
Estrogen	5	164	194	209	202	1.3 ± 0.05	224	518	2.3 ± 0.11
Gonadotropin	5	261	275	295	283	1.1 ± 0.03	580	636	2.0 ± 0.12

"P" value of difference between the effect of the enzyme on the spread of ink in estrogen-treated and gonadotropin = 0.002.

"P" value of difference between the effect of the enzyme on the spread of ink in normal and estrogen-treated = 0.10 (not significant).

"P" value of difference between the effect of the enzyme on the spread of hemoglobin in estrogen and gonadotropin-treated = 0.04.

"P" value of difference between the effect of the enzyme on the spread of hemoglobin in normal and estrogen treated = 0.11 (not significant).

between the skin of estrogen-treated and normal rabbits. This greater hydrolysis of hyaluronic acid by its enzyme in animals under the influence of estrogen than in rabbits under the effects of gonadotropin may be due to a number of variables. The interstitial pressure at the site of injection of the hyaluronidase, the concentration of the substrate, and its resistance to hydrolysis by the enzyme or the modification of the latter by the respective hormones, may be concerned.

A further attempt to differentiate the hyaluronic acid content of the skin of the above three groups of rabbits by staining the skin for metachromasia, as described by Wislocki and Dempsey (17), yielded no significant observations.

THE EFFECT OF ESTROGEN AND CHORIONIC GONADOTROPIN ON TISSUE AND VASCULAR PERMEABILITY

It is established that in the fowl estrogen causes a marked increase in the calcium level of the blood. In the present studies there was a slight increment of

the average concentration of the total serum calcium in estrogen-treated rabbits. Conversely, in rabbits under the influence of gonadotropin these calcium levels were somewhat lower than in control litter mates. However, these effects were not statistically significant. Nevertheless, bearing in mind the work of Chambers and Zweifach (18), which demonstrated that calcium reduces capillary permeability, an attempt was made to determine whether estrogen and chorionic gonadotropin affect this property. The capillary permeability was assayed in an indirect manner, using the method of Menkin (19).

The same group of 15 rabbits used in the above hyaluronidase experiment was tested two days later in the following manner. Three tenths cc. of the supernatant fluid of a centrifuged pleural exudate of one day's duration, derived from a dog treated with turpentine, was injected intracutaneously into each of the 15

TABLE 5

The effect of estrogen and chorionic gonadotropin on tissue and vascular permeability

	NORMAL			ESTROGEN-TREATED				GONADOTROPIN-TREATED			
	Tissue permeability				Tissue permeability				Tissue permeability		
RABBIT NUMBER	Spread of intracutaneous irritant in:		Vascular permeability; intensity of color	Rabbit number	Spread of intracutaneous irritant in:		Vascular permeability; intensity of color	Rabbit number	Spread of intracutaneous irritant in:		Vascular permeability; intensity of color
	15 minutes	1 hour			15 minutes	1 hour			15 minutes	1 hour	
A10=29	182	242	+++	A10=9	171	277	++	A10=11	292	338	+++
A10=42	231	337	+++	A10=31	158	249	++	A10=32	184	275	++++
A10=69	297	404	+++	A10=66	194	226	++	A10=67	219	413	++++
A10=82	225	329	++++	A10=80	151	206	+++	A10=81	147	250	++
A10=71	187	253	++	A10=72	154	212	+++	A10=72	500	626	+++++
Average...	225	313	+++	Average	166	234	++±	Average	269	381	+++±

rabbits in two sites over the chest wall. Immediately thereafter, 15 cc. of a one per cent solution of trypan blue in saline were injected intravenously. Within a few minutes the site of injection of exudate became much more deeply colored than the rest of the skin. This was evidently a consequence of extravasation of dye into the area of increased vascular permeability penetrated by the leukotaxine containing irritant (19). These observations are presented in table 5, where the average of the two areas of extravasated dye in each rabbit is recorded fifteen minutes and one hour after the intravenous injection of the trypan blue. The intensity of the color of the dye at the site of extravasation is recorded as observed two hours after its intravenous administration.

It is apparent that in the estrogen-treated rabbits the exudate spreads over a smaller area than in the control litter mates, whereas in the rabbits under the influence of gonadotropin the irritant spreads over a greater area. This can be explained most readily as the result of the reduced permeability of the connective

tissue in estrogen-treated rabbits on the one hand, and of the increased permeability of this tissue in gonadotropin-treated rabbits on the other.

The intensity of color of these areas of trypan blue extravasation which apparently mirrors the amount of dye exuding from the vessels into the tissues, tends to be less in the estrogen-treated rabbits and more in the gonadotropin-treated animals than in the corresponding litter mate controls. This interpretation is plausible, for this difference could not be accounted for by variations in the rate of removal of dye from the site of extravasation by the tissues, as estrogen would aid its accumulation and gonadotropin would increase its dispersion.

TABLE 6

The effect of estrogen on the circulating lymphocytes of normal and tuberculous inbred rabbit litter mates

GROUP	RABBIT NUMBER	ABSOLUTE NUMBER OF LYMPHOCYTES PER MM³*			
		Before infection		After infection	
		Prior to estrogen treatment of group 2	20 days after estrogen treatment of group 2	11 days after infection	103 days after infection
(1) Not treated with estrogen	C8=2	4,392	4,520	4,579	4,704
	C8=17	4,858	4,185	3,750	5,088
	C8=19	4,772	3,738	5,481	7,627
	C8=23	5,236	3,953	2,525	4,630
	Average...	4,690	4,049	4,084	5,512
(2) Treated with estrogen	C8=1	4,720	2,150	2,208	4,802
	C8=16	4,982	4,683	1,515	1,340
	C8=18	4,862	2,247	1,980	3,032
	C8=24	2,215	2,072	972	2,480
	Average...	4,197	2,788	1,669	2,914

* Per cubic millimeter of free flowing, venous blood.

Thus additional evidence has been given for the view that the permeability of the connective tissue of estrogen-treated rabbits is reduced, whereas that of litter mates under the influence of gonadotropin is increased. Furthermore, there is suggestive indirect evidence that the permeability of the blood vessels may be similarly affected in opposite directions by these two hormones.

THE EFFECT OF ESTROGEN ON THE ADRENALS

During the course of these studies it was found that in estrogen-treated rabbits the absolute number of circulating lymphocytes was usually markedly reduced. This is illustrated in table 6. It will be seen that both in normal and tuberculous rabbits under the influence of the hormone the lymphocytes are conspicuously diminished as compared with those in normal or tuberculous litter mates which were not treated with estrogen.

In view of the demonstration by White and Dougherty (4) that the oxygenated corticosterones, mobilized by the adrenocorticotrophic hormones of the pituitary, cause the dissolution of lymphocytes, it was thought possible that the reduction of circulating lymphocytes in animals under the influence of estrogen may be due to an indirect stimulation of the adrenal cortex by this hormone. Hence studies were undertaken to determine the effect of estrogen on the adrenals.

Twenty-two mice were divided into two groups. Ten animals received three weekly subcutaneous injections of 5 γ of α-estradiol dipropionate in 0.1 cc. of sesame oil. This dose of hormone is equivalent on a body weight basis to that received by the rabbits in which the progress of tuberculosis was markedly retarded by this treatment. Twelve control mice received the same amount of sesame oil without the hormone. Two to four days after the last injection, all the animals were killed, and their total body weight and the weights of their adrenals, thymus, and uterus were determined. Sagittal frozen sections of the adrenals were stained with Sudan III and the width of the cortex was measured in arbitrary units by an ocular micrometer.

To determine the effect of estrogen on the adrenals of rabbits, 5 litter mate pairs, all past the age of eight months, were divided into two groups. One received three weekly subcutaneous injections of 0.5 mg. of α-estradiol diproprionate subcutaneously in 0.5 cc. of sesame oil. The remaining 5 litter mates received the same weekly amounts of oil without the hormoue. Another group of 4 litter mate pairs, seven months or older, were similarly divided and treated with the same amount of estrogen or oil, respectively, at the same intervals, for eight weeks. The first group was killed ten days after the last injection of estrogen. The second group was killed seven days after the last injection of the hormone. As the effect of three or eight weekly injections of estrogen on the adrenal weight was not significantly different, the two groups are treated as one.

To determine the effect of prolonged estrogen treatment on the adrenals of tuberculous rabbits, the weight of these organs at death was measured in 11 litter mate pairs, all simultaneously infected with the same suspension of virulent bovine tubercle bacilli. Each of the pair had been treated with estrogen or sesame oil, respectively, for fifteen weeks or more. The results obtained in these three experiments are summarized in table 7. The average weight of the adrenals is recorded in milligrams per 100 grams of body weight together with the standard deviation of the mean and the "P" value according to the "Student" statistical evaluation for each of the items compared.

It will be seen that the weight of the adrenals of mice given three weekly injections of estrogen is significantly increased. This weight enhancement is due to an increment in the width of the cortex which was 2.2 ± 0.5 micrometer units in the controls, and 2.9 ± 0.19 in the estrogen-treated mice. The difference was statistically significant with a "P" value of 0.002. This confirms the observation of Selye (3). It is noteworthy, as Selye had found, that estrogen causes a marked atrophy of the thymus. The weight of this organ in normal mice was 177 ± 22 mg. per 100 grams of body weight, while that of the estrogen-treated animals was 58 ± 5, with a "P" value of 1.000. Thus, mice given the amount

of estrogen effective in retarding the progress of tuberculosis in rabbits undergo the "alarm reaction" and respond with the general adaptation syndrome as described by Selye (3).

However, rabbits given the same amount of estrogen on a body weight basis for three to eight weekly injections, showed a slight but statistically insignificant increment in their adrenal weights as compared with litter mates treated with the same amount of oil without the hormone. On the other hand, prolonged treatment with this amount of estrogen in tuberculous rabbits is associated with a relative diminution of the adrenal weights as compared with the values for tuberculous litter mates not under the influence of the hormone. This reduction in the adrenal weights is of marginal statistical significance for the "P" value is 0.024. Thus, prolonged treatment with estrogens tends to diminish the size of the adrenals. The meaning of this observation has not been elucidated.

TABLE 7

The effect of estrogen on the weight of the adrenals of normal and tuberculous animals after varying intervals of treatment

NUMBER OF WEEKLY INJECTIONS	ANIMALS USED	NUMBER OF ANIMALS AND THEIR AVERAGE WEIGHT IN GRAMS				WEIGHT OF ADRENALS IN MG. PER 100 GRAMS OF BODY WEIGHT		
		Control		Treated		Control	Treated	"P" value of difference
		Number	Weight	Number	Weight			
3	Mice	12	18.9	10	19.1	38 ± 2.3*	51 ± 3.8†	0.004
3 to 8	Normal rabbits	9	3,390	9	3,170	10.8 ± 1.0	12.5 ± 1.4	0.166
15 or more	Tuberculous rabbits	11	2,320	11	2,630	23.4 ± 2.5	17.1 ± 1.7	0.024

* Width of cortex 2.2 ± 0.05.

† Width of cortex 2.9 ± 0.19. "P" value of difference between the width of the cortex of the adrenals in normal and estrogen-treated mice = 0.002.

There is, however, one striking observation recorded in table 7. The adrenal weight of untreated tuberculous rabbits is more than twice that of normal litter mates, with a "P" value of 1.000. This increase in milligrams of adrenal weight per 100 grams of body weight cannot be ascribed to the loss of body weight due to the tuberculosis and a failure of corresponding loss in adrenal weight. The average loss of weight of the tuberculous rabbits was only 31 per cent, whereas the gain of the adrenals constituted 117 per cent. Clearly, therefore, tuberculosis as such causes an increase in the size of the adrenal and the infection induces an hypertrophy in the adrenal cortex, for the medulla is not affected by the disease. This would suggest that the cortex of the adrenals exercises an important function in the course of tuberculosis. A similar, though statistically less significant, increase in the adrenal weights of estrogen-treated tuberculous rabbits as compared with hormone-treated nontuberculous animals was noted.

Not only is there no constant weight increase of the adrenals in response to estrogen but frozen sections stained with Sudan III of these adrenals, as well as of those of litter mates under the influence of gonadotropin, revealed no consistent differences in the distribution or staining intensity of the lipoids in the several cortical zones of these three groups of animals. Thus, no anatomical evidence of altered function of the adrenal cortex was found on administration of estrogen or gonadotropin.

As adrenal hypertrophy does not regularly follow estrogen treatment in rabbits, the observed reduction in circulating lymphocytes in these animals could not be attributed to an enhanced adrenocortical function. Sayers (20) demonstrated, however, that the corticotrophic hormone of the pituitary markedly reduces the ascorbic acid content of the adrenals, and that the concentration of this substance in the adrenal is a reflection of the corticotrophic funtion. Furthermore, White and Dougherty have caused the dissolution of lymphocytes with corticotrophic

TABLE 8

The concentration of ascorbic acid in the adrenals of normal, estrogen-treated, and gonadotropin-treated litter mates of the inbred rabbit family A

UNTREATED		ESTROGEN-TREATED		GON[illegible]OPIN-TREATED	
Rabbit number	Mg/100 mg. of adrenal	Rabbit number	Mg/100 mg. of adrenal	[illegible]	Mg/100 mg. of adrenal
1	0.094	1	0.075		0.089
2	0.113	2	0.091		0.092
3	0.124	3	0.107		0.098
4	0.133	4	0.120	4	0.106
5	0.152	—	—	—	—
Average.....	0.123 ± 0.019	Average	0.098 ± 0.017	Average	0.096 ± 0.006

"P" value of difference between normal and estrogen-treated = 0.04.
"P" value of difference between normal and gonadotropin-treated = 0.01.

hormone in animals with intact adrenals. Hence it was thought that the concentration of ascorbic acid in the adrenals of control and estrogen-treated rabbits might throw some light on the behaviour of the lymphocytes in these animals.

Accordingly the concentration of reduced ascorbic acid in the adrenals of normal estrogen-treated and gonadotropin-treated litter mates was determined by the method of Rowe and Kuether (21). The results are presented in table 8.

It is evident that the ascorbic acid content of the adrenals of both estrogen and gonadotropin-treated animals is usually less than that of normal litter mates. This would suggest that the adrenals of animals under the influence of either hormone are subjected to increased corticotrophic stimulation. While the data are within the range of statistical significance, they are nevertheless too scanty for definite conclusions.

Further evidence for altered adrenocortical function in rabbits under the influence of these hormones was sought in the glycogen deposition of their livers as this process is a function of the adrenal oxygenated corticocosterones.

For this purpose the livers of the same group of 15 rabbits used in the above experiment on hyaluronidase were stained for glycogen by the Bauer-Feulgen method (22). It will be remembered that these rabbits consisted of three groups of litter mates. Five of these were under the influence of estrogens, 5 had been treated with chorionic gonadotropin, and 5 served as controls. All these rabbits had been on a constant diet for a long time and were killed on the same day. The first group of rabbits had received their third weekly injection of estrogen ten days before death; the second group had received their third intravenous dose of gonadotropin five days before they were killed. The rabbits in these three groups were of uniform weight and nutrition at the time of death, the average weight ranging between 3.1 to 3.4 kg. The estimate of glycogen deposition in each of these rabbits was based on the extent and intensity of the staining reaction.

While there was considerable variation in the deposition of glycogen in the individual rabbits of each of the three groups, it was nevertheless evident that the least glycogen deposits were found in the rabbits under the influence of gonadotropin. In some rabbits under the influence of estrogen the most extensive deposits of glycogen were found, though the overall difference between these rabbits and their litter mate untreated controls was not significant.

Thus no clear evidence has been obtained to demonstrate that estrogen increased the adrenocortical function in the rabbit. It must be stated, however, that in tuberculous rabbits under the influence of estrogen the adrenal cortex always appeared intact. In the adrenal cortex of tuberculous litter mates examined at the same interval after the same inoculation, but not treated with estrogen, there were often areas of necrosis, hemorrhage, and leukocytic infiltration, as may be seen in figures 1 and 2, Plate I.

That the administration of estrogen may affect other glands of internal secretion is suggested by the observation that, in more than half of the tuberculous rabbits under the influence of the hormone, the epithelium of the acini of the thyroid gland was definitely more cuboidal and the colloid accumulation less pronounced than in the untreated tuberculous litter mates.

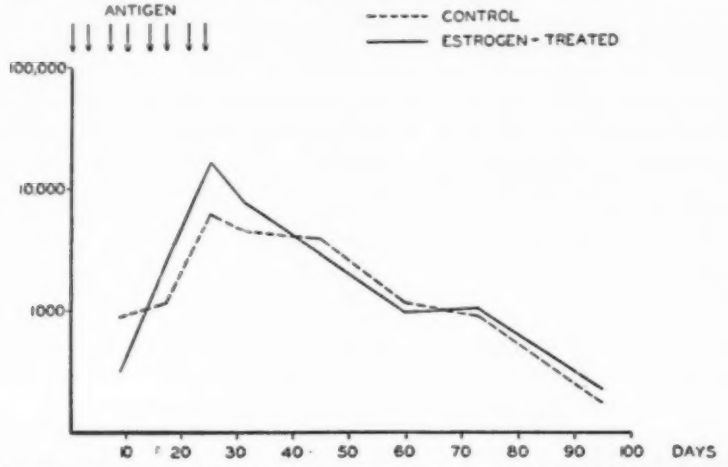
ESTROGEN AND ANTIBODY FORMATION

As stated above, estrogen causes a marked depression of circulating lymphocytes. There is considerable evidence that lymphocytes play a role in antibody formation. It has also been claimed that the administration of oxygenated corticosterones, which cause the dissolution of lymphocytes, enhances antibody formation (23). Moreover, von Haam has reported that theelin increases agglutinin production against the pneumococcus (24). Consequently an attempt was made to determine whether estrogen-treated rabbits produce antibody more rapidly and to a higher titer than normal litter mates.

Three such trials were made, two with chloroform-killed typhoid bacilli as antigen and one with alcohol-killed dysentery bacilli. The results are summarized in figures 1, 2 and 3. Each point plotted in these three figures represents the geometric mean of the antibody titer of 5 rabbits.

It is clear that, irrespective of the number of antigenic stimulations, there is no

evidence that estrogen consistently affects antibody production. There appears to be a slight diminution in the antibody titer of rabbits under the influence



TEXT FIGURE 1. Agglutinin titers in estrogen-treated and control rabbits vaccinated with typhoid bacilli. (Experiment 1. 1947)

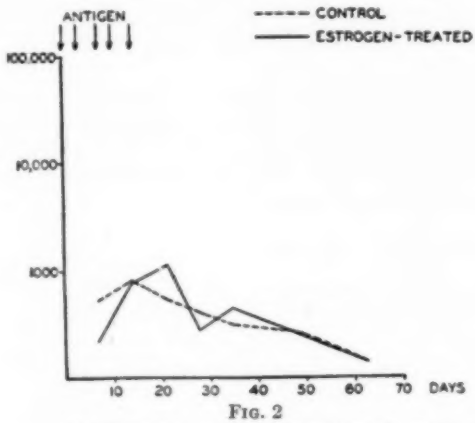


FIG. 2

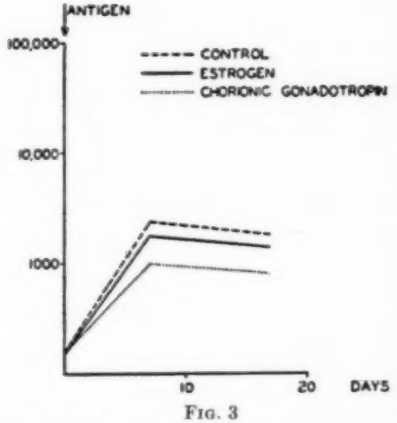


FIG. 3

TEXT FIGURE 2. Agglutinin titers in estrogen-treated and control rabbits vaccinated with typhoid bacilli. (Experiment 2. 1947)

TEXT FIGURE 3. Agglutinin titers in estrogen-treated, chorionic gonadotropin-treated, and control rabbits vaccinated with dysentery bacilli. (Experiment 3. 1948)

of gonadotropin. The difference, however, is not sufficiently constant or pronounced to be significant.

These observations indicate that differences in antibody formation in animals

under the influence of these two hormones do not account for their respective effects on the tuberculous process.

THE EFFECT OF ESTROGEN ON AMYLOID DEGENERATION

As is well known, chronic tuberculosis in man may be associated with extensive amyloid degeneration. Likewise, in rabbits dying from a slowly progressive tuberculosis, there is a severe amyloid degeneration of the malpighian corpuscles of the spleen. Thus the finding that tuberculous rabbits under the influence of

TABLE 9

The effect of estrogen and chorionic gonadotropin on the development of amyloid degeneration and its correlation with the extent of the existing tuberculosis

GROUP	RABBIT NUMBER	EXTENT OF AMYLOID DEGENERATION	EXTENT OF TUBERCULOSIS	GROUP	RABBIT NUMBER	EXTENT OF AMYLOID DEGENERATION	EXTENT OF TUBERCULOSIS
Untreated controls	C6-54	+++	10.0	Estrogen treated	C6-52	0	3.0
	C7-10	+++	20.0		C7-9	0	15.0
	C7-27	+++	17.5		C7-26	+	9.5
	C7-8	+	15.0		C7-7	0	9.0
	C7-29	++++	15.0		C7-28	0	6.5
	C7-13	+++	19.5		C7-12	0,TB*	8.5
	C7-16	+++	13.5		C7-15	±	8.0
	C7-36	+++	27.0		C7-40	+±	16.5
	A10=30	+++	22.0		A10=27	trace	14.0
	A10=23	0	10.5		A10=21	+	19.5
	A10=19	+++	24.5		A10=16	0,TB*	9.0
	A10=26	++	16.5		A10=25	+±	17.5
Untreated controls	A7=29	+++	12.0	Gonadotropin treated	A7=24	+++	17.5
	A8=22	++	16.0		A8=20	+++	16.0
	A8=38	++++	7.0		A7=39	+++	16.5
	A8=45	++++	7.5		A9=56	++	22.0
	A9=54	0	8.0		A9=63	0	18.0
	A9=61	0	13.0		A9=43	+++	9.0
	A9=40	++++	13.0		A9=87	+++	16.0
	A9=44	0	1.5				
	A9=57	0	5.5				

* Tuberculosis present.

estrogen either failed to show any amyloid degeneration at all, or were affected by it only to a very slight degree, appeared to be of considerable significance. Moreover, in untreated litter mates simultaneously infected with the same suspension of virulent bovine tubercle bacilli by the same route, which died or were killed at the same time, the degeneration was usually extensive and frequently had practically replaced most of the splenic parenchyma. This is illustrated in figures 3 and 4 of Plate I.

That this effect is specific for estrogen is indicated in table 9, which records the extent of amyloid degeneration in estrogen-treated and chorionic gonadotropin-

PLATE I

treated tuberculous rabbits, on the one hand, and in their untreated litter mate tuberculous controls, on the other. It will be seen that chorionic gonadotropin far from reducing the extent of amyloid degeneration actually tends to enhance it, whereas estrogen markedly suppresses this tissue alteration. As estrogen tends to retard the tuberculous process and gonadotropin tends to enhance it, it may be claimed that the amyloid degeneration is a result of the extent of the tuberculosis, and that estrogen by suppressing the tuberculosis also suppresses this degeneration. For this reason table 9 includes an estimate of the extent of the tuberculosis in each of these rabbits. This is assigned a numerical value as follows: the severity of the disease in the lung, pleura, and kidney is given a single number which is the sum of the degree of "plus" involvement in each, one plus being assigned the value of one. This sum also includes the number of other organs metastasized. One digit is given to each organ invaded. An examination of this table will reveal that, while there is a certain correspondence between the extent of the tuberculosis and the severity of the amyloid degeneration, the reduction in this degeneration of estrogen-treated rabbits is entirely out of proportion to the diminution of the tuberculosis in these animals.

Discussion

It has been shown in these studies that the administration of large amounts of estrogen to sexually mature highly inbred rabbits retarded the progress of tuberculosis at the site of inoculation in the skin and markedly diminished its dissemination to the internal organs as compared with that in untreated litter mates. In contrast, the induction of successive crops of corpora lutea in the ovary during the early phase of the infection by the periodic injection of chorionic gonadotropin, enhanced the disease at the portal of entry in the skin and increased its spread to the viscera. It has been shown in a previous study (25) that the chief difference in the pathogenesis of the disease in genetically resistant and susceptible rabbits is the degree of localization of the infection. Resistant animals restrict the disease to the portal of entry; susceptible rabbits permit it to spread. Therefore, the present investigation shows that, by altering the internal environment of the tissues of rabbits of the same hereditary resistance to tuberculosis by means of intense exposure to different naturally occurring hormones, one of the fundamental variants in the pathogenesis of the disease in rabbits of different natural resistance has been simulated. However, while there is evidence that

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PLATE I

Magnification of figures 1 and 2 approximately × 440, and of figures 3 and 4 approximately × 100

Fig. 1. (Upper left) Adrenal of control rabbit C7-29 at death. There is a large area of necrosis in the cortex.

Fig. 2. (Upper right) Adrenal cortex of estrogen-treated litter mate C7-28.

Fig. 3. (Lower left) Spleen of control rabbit C7-13. The pulp is largely replaced by amyloid degeneration.

Fig. 4. (Lower right) Spleen of estrogen-treated litter mate C7-12. There is no amyloid degeneration.

in naturally resistant animals the mononuclear phagocytes acquire the power to destroy tubercle bacilli more rapidly and effectively than the cells of susceptible animals, estrogen does not affect the growth and destruction of the bacilli in the tissues.

Since it was found in the study cited (25) that the most resistant family acquired allergic sensitivity on stimulation with heat-killed tubercle bacilli more rapidly and intensely than the most susceptible family, the effect of estrogen and chorionic gonadotropin on tuberculin sensitivity was studied. It was found that estrogen markedly suppressed the inflammatory response of the skin to tuberculin in rabbits which had been sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli. However, this seemingly depressing effect of estrogen on allergy was confined to the skin. The extent of caseation in the internal organs, the leukocytic response to the subcutaneous injection of tuberculin, and the sensitivity of the cells to tuberculin in tissue culture were not diminished by the hormone. Furthermore, even the lowered skin sensitivity to tuberculin could be restored by withdrawal of the hormone. The underlying allergizing mechanism was not affected by estrogen. The hormone merely masked an existing undiminished allergic skin sensitivity. Estrogen reduces the inflammatory response of the skin to bacterial toxic agents in general and, among these, to tuberculin, which is toxic to sensitized animals. Furthermore, in experiments not detailed it was found that chorionic gonadotropin did not affect the rate or intensity of development of allergic irritability of inbred rabbits treated with heat-killed tubercle bacilli.

Thus the mechanism whereby estrogen tends to localize the infection and gonadotropin tends to spread it differs from that by which naturally resistant rabbits restrict the infection to the portal of entry and susceptible animals permit its dissemination.

It was noted above that ovariectomy, which has no effect on the tuberculous process, and gonadotropin, which enhances the disease, both are associated with a lower skin sensitivity to tuberculin during the course of the infection. Therefore, the lower inflammatory irritability of the skin of estrogen-treated rabbits to tubercle bacilli and their products does not account for the retarding effect of the hormone on the disease.

It has been shown in these and previous studies that the spread of particles in the connective tissue is reduced by estrogen and increased by gonadotropin. In order to determine the mechanism whereby estrogen reduces the permeability of the connective tissue and thus hinders the dissemination of the disease from the portal of entry, the water content of the skin was investigated. It was found that the total concentration of water in estrogen-treated rabbits was not increased. However, the distribution of the water between the extra- and intracellular compartments was not assayed. Taylor and Sprunt determined the extracellular fluid of the skin of rabbits under the influence of this hormone by the thiocyanate method (26) and found a significant increase. This would suggest that, as a result of the swelling of the connective tissue elements caused by estrogen, their turgescence is increased and their permeability to particles reduced.

That the tautness of the connective tissue may be an important factor in its permeability was suggested by the following observation. Hyaluronidase enhanced the spread of particles to a greater extent in the skin of estrogen-treated rabbits than in that of litter mates under the influence of gonadotropin. As stated in the body of this paper, this may be due to a number of variables but the simplest explanation is afforded by the work of Hechter (16). He demonstrated that the spreading effect of hyaluronidase is determined not only by its concentration but also by the volume and pressure of the intradermal injection. The greater the interstitial pressure at the point of introduction of the enzyme, the more widely will the enzyme spread in the tissues, and the greater will be the extent of the hydrolysis of the hyaluronic acid matrix of the skin. As estrogen reduces permeability and gonadotropin enhances it, it is clear that a given volume of fluid injected into the skin of an estrogen-treated rabbit will be under a greater pressure than in an animal under the influence of the gonadotropin. Therefore, the hyaluronidase will diffuse to a greater degree in the estrogen-treated than in gonadotropin-treated animals. On this basis, no assumption need be made as to the effect of these hormones on the enzyme or its substrate to explain the observations. Therefore it may be inferred that one factor in the permeability of the connective tissue is the turgidity of its elements.

Finally, by the use of Menkin's technique presumptive evidence was obtained which suggests that the vascular permeability of the skin may be reduced by estrogen and increased by gonadotropin. All these observations are in harmony with the observed restricting effect of estrogen on the dissemination of the disease from the portal of entry in the skin and its enhancement by gonadotropin.

Physiologic doses of combined progesterone and estradiol, which also enhance the spread of particulate matter in the skin, do not increase the dissemination of tuberculosis in the body. This would suggest that the action of corpora lutea engendered by gonadotropin was not completely simulated by the administered hormones. On the other hand, the failure of estrogen to restrict the tuberculosis in sexually immature rabbits regularly would imply that other changes besides reduced connective tissue permeability may play a role in this hormone's capacity to retard the disease.

It has been shown by Selye that folliculoids induce the adaptation syndrome. In mice, the administration of estrogen in amounts comparable to that used in rabbits induced hypertrophy of the adrenal cortex and atrophy of the thymus. In rabbits there is a reduction of circulating lymphocytes but no increase in adrenal weights as a result of estrogen. Moreover, no definitive evidence was obtained by the study of liver glycogen deposits of increased adrenocortical function in the rabbit as a result of estrogen treatment. Lipoid stains of the adrenals of these animals also failed to indicate any alteration of cortical function.

While the adrenals of rabbits show no hypertrophy in response to estrogen treatment, they nevertheless appear to contain less ascorbic acid than the adrenals of untreated litter mates. This fact suggests that the glands are subjected to increased corticotrophic stimulation of the pituitary. This acid was also reduced, however, in animals under the influence of gonadotropin. Hence the opposite effects of these two hormones on the tuberculous process cannot be

ascribed to their adrenocortical influence on this basis. The increased secretion of ascorbic acid by the adrenals may be a factor in the reduction of the tuberculin sensitivity of their skin, for Steinbach (27) showed that sensitized guinea pigs may be protected from tuberculin shock with ascorbic acid.

In view of recent claims that antibody production is enhanced by the administration of cortical hormones, it is noteworthy that neither estrogen nor gonadotropin significantly affected agglutinin formation against the typhoid or dysentery bacillus. While there is no clear evidence that these sex hormones affect the disease via the adrenals, it must be emphasized that tuberculosis as such causes a marked hypertrophy of the gland, suggesting that it plays a significant role in the tuberculous process. In this relation it may also be stated that in one-half of the rabbits under the influence of estrogen there was some hyperplasia of the thyroid. This indicates that the administration of estrogen is not without effect on other endocrines.

One of the most impressive differences between estrogen-treated and control tuberculous rabbits was the marked reduction in amyloid degeneration in the spleen which is characteristic of rabbits dying from chronic tuberculosis. This could not be accounted for by the less extensive disease in the hormone-treated animals. Frequently the difference in the extent of the disease was only moderate in the two litter mates, whereas the amyloid degeneration was extensive in the control animal and completely absent in the estrogen-treated rabbit. This effect was specific for estrogen, as ovariectomy or progesterone and gonadotropin treatment had no effect on the degree of amyloid degeneration. The significance of this observation remains to be determined.

In the same category is the fact that, while prolonged estrogen treatment tends to reduce the adrenal weights, the incidence of degenerative processes in the adrenal, such as hemorrhage, leukocytic infiltrations, and focal necrosis is definitely reduced in estrogen-treated rabbits. What this means has not yet been ascertained.

Conclusions

1. Estrogen retards the progress of tuberculosis at the portal of entry in the skin and diminishes its dissemination to the internal organs in highly inbred rabbits chiefly by reducing the permeability of the connective tissue.

2. Chorionic gonadotropin enhances the disease at the portal of entry and its spread through the body mainly by increasing the permeability of the connective tissue.

3. Hyaluronidase exerts a greater spreading effect in estrogen-treated animals than in rabbits under the influence of gonadotropin.

4. Estrogen reduces the number of circulating lymphocytes of the blood. The intermediation of the adrenal cortex in this effect was not demonstrated.

5. Tuberculosis *per se* induces a marked hypertrophy of the adrenals in rabbits.

6. The reduction of the inflammatory responsiveness of the skin to the products of tubercle bacilli induced by estrogen is not a significant factor in this hormone's capacity to retard the tuberculous process.

7. Estrogen and gonadotropin exert no effect on antibody formation.

8. Estrogen markedly suppresses the development of amyloid degeneration in the spleens of tuberculous rabbits.

Sumario

Factores Constitucionales en la Resitencia a la Infección: III. Modo de Actuar del Estrógeno y de la Gonadotropina sobre la Evolución de la Tuberculosis

1. El estrógeno retarda el proceso tuberculoso en la vía de entrada en la piel y disminuye su diseminación a los órganos internos en los conejos muy entrecruzados, principalmente por reducir la permeabilidad del tejido conjuntivo.

2. La gonadotropina coriónica realza la enfermedad en la vía de entrada y su propagación por el cuerpo, principalmente por acrecentar la permeabilidad del tejido conjuntivo.

3. La hialuronidasa ejerce mayor efecto propagador en los animales tratados con estrógeno que en los conejos sometidos al influjo de la gonadotropina.

4. El estrógeno reduce el número de linfocitos en la circulación. No se demostró la intervención de la corteza suprarrenal en este efecto.

5. La tuberculosis evoca *per se* pronunciada hipertrofia de las suprarrenales en los conejos.

6. La disminución, evocada por el estrógeno, de la respuesta inflamatoria de la piel a los productos de los bacilos tuberculosos no constituye un factor significativo en la capacidad de dicha hormona para retardar el proceso tuberculoso.

7. El estrógeno y la gonadotropina no ejercen efecto alguno sobre la formación de anticuerpos.

8. El estrógeno suprime notablemente el desarrollo de degeneración amiloidea en los bazos de los conejos tuberculosos.

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LETTERS TO THE EDITORS

STREPTOMYCIN-DEPENDENT STRAINS OF MYCOBACTERIUM TUBERCULOSIS

To the Editors of the American Review of Tuberculosis:

Miller and Bohnhoff in 1947 (Science, 1947, *105* (2737), 620) were the first to demonstrate that bacterial variants dependent upon streptomycin for growth may appear in cultures otherwise sensitive to streptomycin. This observation has been confirmed since by a number of other investigators. Recently we have been successful in isolating several streptomycin-dependent variants of *M. tuberculosis*.

In a study of the chemotherapeutic value of various antituberculous agents, mice infected intracerebrally with the $H_{37}Rv$ strain of *M. tuberculosis* were cultured at the time of death on four media: (*1*) Dubos liquid medium, containing 0.02 per cent Tween 80 and 0.5 per cent albumin; (*2*) Dubos medium to which had been added streptomycin in a concentration equivalent to 500 γ of pure streptomycin base per ml.; (*3*) Herrold's egg yolk agar; and (*4*) Herrold's egg yolk agar to which streptomycin had been added in a concentration equivalent to 500 γ of pure base per ml. Incubation was carried out at 37° C for a period of six to eight weeks.

A total of 196 animals were cultured. Of these, 161 were positive for tubercle bacilli when cultured on the Dubos medium containing no streptomycin; 147 were positive when cultured on Herrold's egg yolk medium containing no streptomycin. On one or both of these media, 184 (95 per cent) of the 196 animals cultured were positive for tubercle bacilli. All of these strains were highly sensitive (<10 γ per ml.) to streptomycin in spite of the fact that many of the animals had received repeated injections of large dosages of streptomycin for thirty-one days following inoculation.

Of the 196 animals cultured, 13 were positive for tubercle bacilli when cultured in Dubos liquid medium containing 0.02 per cent Tween 80, 0.5 per cent albumin, and the equivalent of 500 γ of streptomycin base per ml. These same 13 animals all showed growth of streptomycin-sensitive tubercle bacilli when cultured in the absence of streptomycin. All of the strains isolated on the streptomycin-containing media at first grew only in the presence of high concentrations of streptomycin. On subsequent transfers *in vitro*, rapid and heavy growth appeared in all instances on media containing streptomycin, dihydrostreptomycin, mannosidostreptomycin, or streptomycin residues containing both streptomycin and mannosidostreptomycin. Faint growth occurred at this time, however, in all instances on streptomycin-free media, but subculture of these nondependent variants indicated that they could not be propagated repeatedly in the absence of streptomycin.

In all cases, the streptomycin-dependent strains of tubercle bacilli described above were highly resistant to streptomycin *in vitro*. The sensitivity of 8 of these strains to streptomycin ranged from 5,000 to 10,000 γ per ml., while 2 were sensitive to 2,500 γ per ml. The remaining 3 strains grew readily in concentrations of streptomycin equivalent to more than 500 γ of pure streptomycin base per ml. In all instances, the growth was less in the presence of low concentrations of drug, suggesting that the strains may well contain some cells growing more readily in the presence of higher concentrations of streptomycin.

In no instance were streptomycin-dependent variants isolated on Herrold's egg yolk medium containing streptomycin.

Of the 13 streptomycin-dependent strains isolated, 6 were tested for virulence and for their *in vivo* sensitivity to streptomycin. Mice belonging to the C57 or DBA strains were

infected intracerebrally with 0.01 cc. of a standardized culture of each strain. All cultures used for inoculation of mice were grown for ten days in the Dubos liquid medium containing 0.02 per cent Tween 80 and 0.5 per cent albumin. Cultures were diluted with sterile Dubos liquid medium immediately prior to use to a final density of 57 to 63 per cent transmission (Photovolt Lumetron No. 400). A portion of the infected mice were held as untreated controls while 10 received 4 mg. (total weight) of pure streptomycin sulfate daily by the subcutaneous route for a period of thirty-one days. All animals were observed for a period of six months. Animals were autopsied at the time of death and the presence of tuberculous lesions determined by gross pathological examination, culture for tubercle bacilli and by microscopic examination for acid-fast bacilli. The degree of pathogenicity was measured by the rate at which the mice succumbed to the tuberculous infection. Only one or 2 of the 6 strains showed a degree of virulence comparable with the original culture of $H_{37}Rv$ from which these streptomycin-dependent strains had been derived. The remaining strains possessed little virulence for mice, three to six months being necessary to produce death in a portion of the animals, while the remainder survived for a period of over six months. In no instance was virulence enhanced by the administration of streptomycin. Those strains possessing virulence, however, were sensitive to streptomycin *in vivo*.

At the time of death all animals showed gross evidence of tuberculosis. On culture from the lungs of a portion of these animals, both streptomycin-resistant and streptomycin-dependent strains of tubercle bacilli were isolated. In no instance were streptomycin-sensitive strains recovered. This is of interest in view of the fact that some of the cultures were recovered from streptomycin-treated animals in which the per cent survival was slightly greater than in the nontreated animals.

It is apparent from these observations that variants of *M. tuberculosis* dependent upon streptomycin for growth may appear in otherwise sensitive cultures. It is further apparent that dependency probably is associated closely with resistance in view of the fact that all dependent strains isolated were resistant to streptomycin *in vitro*, and on passage *in vivo* produced streptomycin-resistant and streptomycin-dependent strains only. The ability of microorganisms to adapt themselves in such manner as to permit their utilization of streptomycin as an essential factor for growth may contribute in part to the limited effectiveness of the drug as a chemotherapeutic agent.

T. F. Lenert
G. L. Hobby

Biological Laboratory
Chas. Pfizer & Co., Inc.,
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TUBERCULOSTATIC ACTIVITY OF AUREOMYCIN IN VITRO AND IN VIVO

To the Editors of the American Review of Tuberculosis:

In view of the interest in the possibilities of aureomycin as a therapeutic agent in tuberculosis, it may be of value to present some preliminary results of experiments on the tuberculostatic effect of this drug *in vitro* and *in vivo*.

The growth inhibiting action of aureomycin[1] (Lederle Lot No. 7-8071 A, crystalline hydrochloride) on the laboratory strain H37 Rv was tested in four different types of fluid medium. After eleven days of incubation, growth was inhibited by 5 γ of aureomycin per cc. in Tween-albumin medium; 2.5 γ per cc. in Proskauer and Beck's medium; 5 γ per cc. in Youmans' modification of the Proskauer and Beck medium, and 40 γ per cc. in the latter medium plus 10 per cent beef serum. At the sixteen day observation period, the minimal inhibitory concentration was 10 γ per cc. in Tween-albumin and Proskauer and Beck's medium, 20 γ per cc. in Youman's modification of Proskauer and Beck's medium, and over 40 γ per cc. in the medium with serum.

Twenty guinea pigs (average weight 500 Gm.) were infected subcutaneously with 0.02 mg. of the H37 Rv microorganisms and one-half of the group were started on aureomycin treatment ten days later. The drug was given by intramuscular injection at twelve hour intervals. Initial dosage was 0.2 mg. per day, which was increased gradually to 1.6 mg. per day. It was felt that the animals could not tolerate much more than this amount for they exhibited a marked weight loss and appeared to be in worse condition than the untreated controls subsequent to the third week of therapy.

All survivors were killed 58 days after infection. At this time, 7 of the treated animals had died, but only 2 of the controls had succumbed. A comparison of the amount of tuberculous disease observed grossly at death or sacrifice revealed no difference between the group treated with aureomycin and the untreated control group.

It would appear from these observations that aureomycin, in the dosage used, had no deterrent effect on the course of the tuberculous disease in guinea pigs.

It is realized that the aureomycin dosage was relatively small, but it compares favorably with the intramuscular dosage in man reported in the literature (0.6 to 3 mg. per kg. per day, Bryer *et al.*, J. A. M. A., 1948, *138:* 117; and Wright *et al.*, J. A. M. A., 1948, *138:* 408). Moreover, the dose used in the present experiments was the maximum tolerated dosage for the guinea pigs by this route. The response of tuberculous infection of animals of other species which might be able to tolerate higher doses of aureomycin cannot be predicted. The intramuscular administration of aureomycin to guinea pigs, however, had no effect upon the course of their tuberculous infections.

THE TRUDEAU LABORATORY W. STEENKEN, JR.
TRUDEAU, NEW YORK E. WOLINSKY

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ABSTRACTS

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Tuberculous and Meningococcal Meningitis.—A patient with meningococcal meningitis was being treated adequately. Although the cerebrospinal fluid became sterile, the patient's condition suddenly took an unfavorable turn. Evidence of a coexisting tuberculous infection then was found and the patient died. The diagnosis was confirmed by necropsy. In retrospect, the fact that only 54 per cent of the cells in the cerebrospinal fluid were neutrophiles is of interest. The reported case is about the thirty-fourth in the literature.—*Coexisting tuberculous and meningococcal meningitis, E. A. Riley, New England J. Med., September 29, 1948, 239: 386.*—(A. G. Cohen)

Tuberculous Pericarditis.—Tuberculosis is recognized as a cause of constrictive pericarditis. Pockets of fluid or of inspissated residue have been found in the pericardium but a frank abscess containing tubercle bacilli has not been reported. Pleural effusion is a frequent concommitant and may be part of a polyserositis. A case is reported which progressed through the usual course of pericardial effusion, pleural effusion, and venous obstruction. At operation a tuberculous abscess was found, pericardectomy was done, and streptomycin was instilled locally and given parenterally after operation. A good clinical result was obtained and no sinus developed.—*Constrictive pericarditis with tuberculous intrapericardial abscess treated with streptomycin, J. A. Moore & J. D. Murphy, Ann. Surg., May, 1948, 127: 685.*—(*W. H. Oatway, Jr.*)

Pathogenesis of Industrial Pulmonary Disease.—The discussion is limited to subacute and chronic pulmonary disease resulting from the inhalation of particulate matter of low solubility. The inhalation of irritating dusts results in certain changes in the behavior of the lung which in some cases favor the retention of fine particles in larger numbers than usual. For example, there is a reduction in the pulmonary volume and an increase in the rate accompanied by bronchiolar constriction which, if maintained, leads to stasis of secretions, patchy atelectasis, and emphysema. Bronchiolar constriction is accompanied by an increase in residual air. Reduction of the capillary flow in atelectatic areas creates conditions favorable to the accumulation of phagocytes which have engulfed the dust. These phagocytes may die *in situ*, autolyze, and liberate dust which, if toxic, stimulates local fibrosis. Thus atelectasis and emphysema resulting from bronchiolar constriction favor markedly the local deposition of dust. The majority of the particles are carried by lymphatics to the hilar nodes but some enter the capillaries and flow into the systemic circulation. This is a protective mechanism as regards particles that have settled in the alveoli and the overall drainage rate is an important factor. The upper portions of the lung are first and most severely affected, suggesting that the rate of drainage is closely related to lung movement. This is consistent also with the observation that continued activity sometimes delays the onset and lessens the disability in some progressive pulmonary diseases. At present the solubility of the

particles is thought to be the most important factor in the production of pneumoconiosis. Certain critical considerations must be evaluated, however, before accepting this theory. (*1*) The chemical attributes of inhaled material must greatly affect the character and degree of tissue reaction. (*2*) The differences in solubility between active and inactive dusts have been classified only empirically as yet. (*3*) Toxic materials and ions which dissolve from the surface must be shown to differ quantitatively and qualitiatively and their solubility must be related to the pathogenicity of the particles. This had been shown to be generally true of silica. The determination of solubility of silicious materials is difficult but it has been shown that the solubility of quartz dust is inversely related to the particle size. Other unsolved problems include the effects of critical concentration on the rate and the course of development of the disease. It follows that the development of chronic pulmonary disease is dependent upon (*1*) the chemical characteristics of the dust, (*2*) the solubility rate of particles within certain ranges and (*3*) exposure to an adequate concentration of dust over a sufficient period of time. The rate of development will depend upon the adequacy of the drainage system as determined by previous history with respect to earlier infections and exposure to the irritant. Tissue reactions are seen peribronchially, in the pleura, perivascular tissues, interstitial tissues, alveolar wall, and along the lymphatics. Where phagocytes have accumulated and died, focal proliferation in the perilymphatic sheaths with an accumulation of phagocytes impairs lymphatic drainage. The outstanding pathology may be in the lung and the disturbance of lung function may dominate the picture but with long-continued, severe exposure lesions develop in the abdominal viscera.—*Pathogenesis of industrial pulmonary disease, W. Machle, Radiology, June, 1948, 50: 755.*—(*G. F. Mitchell*)

Pulmonary Changes in Beryllium Workers.—Workers are exposed to beryl dust at the mines during the various processes of extraction of beryllium from the ore, the production of its salts, oxides and alloys, during the manufacture of fluorescent lamps, neon tubes, radio tubes, and in research and pharmaceutical plants. In all of these situations there has been definite evidence of disease among the employees with symptomatology, roentgenographic pattern, and pathology so similar that beryllium or one of its compounds is suspected as the cause. The incidence and severity of the disease are proportionate to the degree of exposure and chemical irritation. Most patients recover within a few weeks or months following removal from exposure but recurrence may occur without re-exposure and the disease may last months or years. The onset may be as long as six years after the last exposure. The onset is insidious and characterized by cough with occasional dull, burning substernal pain. Dyspnea follows the cough; cyanosis, low blood pressure and clubbing of the fingers are frequent physical findings. Anorexia and weight loss are common, usually accompanied by increasing fatigue. Physical findings indicate an absence of infection although the pulse rate is rapid. The pathology is that of an atypical pneumonitis, pulmonary edema, and hemorrhage. The lesions in the so-called chronic pulmonary granulomatosis are seen grossly as small pinhead-sized, whitish nodules throughout the lungs. Lymph nodes occasionally are enlarged and may show areas of granular, grayish-white necrosis. The earliest lesions consist of clumps of hystiocytes and moderate edema within the alveoli. Later the nodules of histiocytes are surrounded by a zone of lymphocytes and occasional plasma cells. The end stage is marked by an area of hyalinization. Only subacute and chronic forms of the disease have been recognized but it is possible that there is an acute type also. The roentgenographic findings are dependent upon the pathology present. A diffuse ground glass density appears in both lungs one to three weeks after the onset of symptoms, followed by areas of soft infiltrations accompanied by distinct linear markings and hilar enlargement. The infiltrations disappear and small

nodules are then seen. These disappear in one to four months and are usually gone before the symptoms subside. Some residual fibrosis may be seen. Most of the cases followed by the authors, some since 1942, were of the chronic type. Much hilar lymph node enlargement was found in these cases and was associated with upward displacement of the short septum and with basal emphysema. With the increase in basal emphysema more cases having clubbing of the fingers and cyanosis may appear. Seven cases developed small pneumothoraces. A few cases have shown a clearing of the granular and nodular densities, but some residual linear fibrosis was present and clinically there was evidence of subnormal pulmonary function. The size of the nodules varied in individual cases; in a few the original "sand-like" pattern was retained or simulated by a fine nodulation. Roentgenograms during acute exacerbations showed what is believed to be edema. The patient usually recovered with bed-rest and oxygen therapy and the roentgenographic findings returned to the pattern seen before the attack. Those who succumbed usually had cor pulmonale. The degree of involvement as seen roentgenologically is not always an indication of the degree of disability. Differential diagnosis includes a consideration of pulmonary sarcoidosis, miliary tuberculosis and silicosis. No specific mode of treatment has been found. Spectrography has been the only reliable method for determining such small amounts as 5 γ or less. It can be used to detect beryllium or beryllium products in the urine and to analyze dust concentrations in the plants.—*Delayed chemical pneumonitis or diffuse granulomatosis of the lung due to beryllium, S. A. Wilson, Radiology, June, 1948, 50: 770.*—(*G. F. Mitchell*)

Beryllium Pneumonitis.—A laboratory. worker developed a chemical pneumonitis three years after exposure to zinc beryllium manganese silicate used in making fluorescent lamps. The period of exposure was one year. The chief symptoms were cough, dyspnea and extreme loss of weight. The roentgenogram four months after the onset showed fine punctate lesions or gray markings throughout both lung fields. A film at eight months showed the same findings with some evidence of a reticular pattern. At seventeen months, there was more reticulation with small scattered nodules about 2 mm. in diameter. At twenty-one months, there was more definite fine nodulation. At thirty months, there was considerable fibrosis. A liver biopsy showed a granulomatous lesion. Clinically, there was no improvement three years after the onset.—*Delayed pneumonitis in a beryllium worker, J. N. Agate, Lancet, October 2, 1948, 2: 530.*—(*A. G. Cohen*)

Anthraco-silicosis.—Anthraco-silicosis, commonly called "miner's asthma", is a chronic disease due to breathing air containing dust formed in the various processes of mining and preparing anthracite coal. It is characterized anatomically by generalized fibrotic changes throughout the lungs, the presence of excessive amounts of carbonaceous and siliceous material, a compensating emphysema, and often by cardiac changes in the later stages of the disease. A review of some observations made in coal miners with anthraco-silicosis over a period of 27 years is presented. One thousand cases were studied from the standpoint of the age and build of the worker, the working conditions, years of exposure, and race. No race was found to be particularly resistant. A man must be exposed to a siliceous dust from five to seven years before changes in the lungs can be noted in the roentgenogram. A thorough history and roentgenographic examination, including fluroscopy, is essential for the diagnosis. Stereoscopic films are indispensible in the first and second stages of the disease. Infection is more frequent in the third stage of the disease and nonspecific infections in the first and second stages respond quite well to ordinary treatment. Tuberculosis is not a common complication in the first two stages of the disease nor under the age of fifty. Total and permanent disability has never been observed from anthraco-silicosis alone unless the dis-

ease has advanced well into the third stage. Evidence so far seems to indicate that the disease does not progress to the third stage if the patient is completely removed from exposure to the dust. Since anthraco-silicosis is a progressive, incurable disease, the only way to counteract it is to remove the silica from the atmosphere in which the men work. The men cannot be removed without closing the industry. The best method for removing the siliceous dust is a system of water sprays used before and after drilling and blasting.—*Anthraco-silicosis, W. J. Corcoran, Radiology, June, 1948, 50: 751.*—(*G. F. Mitchell*)

Lung Changes from Alumina Abrasives.—A review of the case histories of men who have been employed in the manufacture of alumina abrasives shows that there are now 34 well established and 38 early cases of distinctive pulmonary disease associated with this industry. The classification of well established disease is based on clinical and radiological evidence. All patients with symptoms have changes of moderate to extreme degree demonstrable by roentgenography. Others may have few symptoms but in each the roentgenogram shows a widened mediastinum with considerable parenchymal shadowing; such persons usually progress to a more serious type of disease. The longest period of exposure reported was nineteen years, the shortest eighteen months. The men who change the furnace pots and instructors had greater exposure to the hazard than binmen and cranemen. Several men in various plants have worked as long as nineteen years continuously as furnace feeders and have shown no roentgenographic changes to suggest that they are suffering from the disease. Disease from working in alumina abrasives was first discovered during the recent war but there is sufficient evidence to show that the disease was present prior to that time. A review of previous films has uncovered additional cases which have been classified as showing early disease. These were not previously reported. Seven of 34 well established cases died within a relatively short time after they were first seen. Three cases have been diagnosed and the men removed from exposure too recently to evaluate the results. The remaining 24 cases are presented as showing extension of the disease in 4 different degrees with distinctive roentgenographic changes. There is little evidence to prove that acute infection plays any major part in the progression of the disease.—*Further observations of lung changes associated with the manufacture of alumina abrasives, C. G. Shaver, Radiology, June, 1948, 50: 760.*—(*G. F. Mitchell*)

Acute Pulmonary Actinomycosis.—A twenty-year-old woman was successfully treated for acute pulmonary actinomycosis complicated by actinomycosis of the right pleural cavity. The pneumonic process appeared to respond to intramuscular penicillin, but this did not prevent the formation of empyema. After actinomyces had been identified in the pleural pus, penicillin by intramuscular injection was continued, together with sulfonamide therapy ("Elkosin" by mouth) and the pus was drained through an intercostal incision. Improvement from this point on was prompt but there was recurrence of empyema which necessitated drainage through a higher space. Convalescence then proceeded uneventfully and the patient has remained without recurrence for one year. In all, 1,425,000 units of penicillin and 250 Gm. of "Elkosin" were given. Local treatment of the empyema space with penicillin was not carried out although a review of the literature shows that this is effective in more chronic cases.—*Heilung eines Falles von Lungenaktinomykose mit postpenumonischem aktinomykotischem Empyem durch Bülau-Drainage und Penicillin-Elkosin Medikation, R. Fink, Schweiz. med. Wchnschr., January 10, 1948, 78:19.*—(*M. Marcus*)

Q-Fever.—Up to 1947 Q-fever had not been identified in Switzerland. Undoubtedly cases of virus-type pneumonia might have been diagnosed as such in the past if proper methods of identification of the rickettsial disease had been available. The author reports cases

observed in 1947 which could be proved to be identical with Q-fever by complement fixation tests. He was able to identify the disease among a family group of 4 people all of whom became ill within three days with a characteristic febrile illness accompanied by moderate to marked prostration and extensive pathological findings in the lungs. Another epidemic of this disease was obsered in a village where 50 cases of Q-fever pneumonitis were seen within four weeks. One of these cases was fatal. Thirty cases were observed in a boarding school in Geneva in June, 1947 and sporadic cases have been observed in the spring and fall of 1947 in the canton of St. Gallen. The illness was characterized by fever, severe constitutional symptoms, headache, and profound prostration. Physical examination was often negative but roentgenograms usually showed impressive consolidations in one or more lobes. The course was usually benign with resolution between the fifth to twelfth day, but pleural effusions and orchitis were frequently seen. The etiological agent is Rickettsia burneti and diagnosis is usually made by rising titers of the specific complement fixation test. A titer of more than 1:28 is diagnostic. The vector is believed to be Dermacenter andersoni which is commonly found on cattle but ticks were not encountered in the cases reported in this paper. Treatment of the illness is supportive and symptomatic.—*Q-fever (Queenslandfieber) in der Schweiz, O. Gsell, Schweiz. med. Wchnschr., January 10, 1948, 78: 1.*—(*M. Marcus*)

Bronchial Adenoma.—Of 217 primary bronchial tumors proved histologically at the Lahey Clinic since 1930, 15 (6.9 per cent) were adenomas. They were located principally in the major bronchi. They were seen by the bronchoscopist in 12 of 14 cases examined. Grossly the tumor appears as a rounded, pink or reddish-purple mass, usually attached by a broad base. It bleeds profusely with manipulation. There are 2 histological types, carcinoid and mixed (cylindroma); the latter is considered by some to have malignant potentialities. Cases of adenoma which underwent malignant degeneration with or without metastases have been reported. Local infiltrations are frequent. Sixty per cent of the cases were in patients less than 40 years of age. Eight were in females. The duration of respiratory symptoms prior to diagnosis ranged from eight months to six years. Cough is the most frequent symptom; it usually becomes progressively worse and eventually is productive. Hemoptysis is an outstanding symptom. A wheeze was found in 3 cases. Dyspnea was present in 4 cases and was the result of atelectasis, obstructive emphysema or mediastinal shift. Varying degrees of chest discomfort were noted and pulmonary infections were frequent. Abnormal physical signs varied with the pulmonary changes. The treatment is either local removal or pulmonary resection. Local removal is adequate provided that there is a pedicle and no extrabronchial extension, irreversible pulmonary damage or involvement of the carina.—*Bronchial adenoma, C. R. Souders & J. W. Kinasley, Jr., New England J. Med., September 23, 1948, 239: 459.*—(*A. G. Cohen*)

Calcification in Twins.—Pulmonary calcification in ten-year-old twin sisters is reported. Their skin tests were negative with tuberculin and coccidioidin, but positive with histoplasmin. They had lived in Nebraska, Colorado, Illinois, Kansas, California and Michigan, several of which are in the Mississippi basin area where histoplasmosis is believed to be most prevalent. The child with the greater amount of calcification had previously had asthma, had a low grade fever, but had no current lung symptoms. Studies of sputum and nodes produced no fungus.—*Pulmonary calcification in twins, G. Houston & W. A. Steiger, J. Pediat., June, 1948, 32: 706.*—(*W. H. Oatway, Jr.*)

Streptomycin for Tuberculous Meningitis.—The results of treatment of 72 cases of tuberculous meningitis are recorded; 13 of these began as miliary tuberculosis. In 9 cases no streptomycin was given and death ensued. The drug was given in 63 cases, of whom 34

died. It was administered both intramuscularly and intrathecally. The dosage was 1.0 Gm. daily or less. The more severe the signs, the smaller should be the intrathecal dose. The average duration of treatment was three to four months. Continuous treatment was given initially for four to six weeks, resulting in amelioration of signs and symptoms, but in persistence of the laboratory evidence of infection. After a lapse of five to ten days, treatment was resumed for ten to fifteen days. This schedule of intermittent treatment was continued until there were no laboratory signs of infection. As long as the cell count and chemical findings remained abnormal, the dangers of exacerbation were great. By lengthening the duration of the disease and prolonging life, the treatment increased the incidence of hydrocephalus. The irritating effects of large doses of streptomycin contributed to the development of this complication. Thus, in the series treated with streptomycin more of the fatalities were due to hydrocephalus than in previously untreated cases. There is less risk of hydrocephalus in cases in which treatment is begun early. Of 50 treated cases of tuberculous meningitis, 21 were cured, 3 are still under treatment and 26 died. The survivors were usually the ones in whom treatment was started in less than ten to fifteen days after the onset of the disease. In the fatal cases, the length of survival was longer than in untreated cases. In 13 cases of miliary tuberculosis complicated by meningitis, there were cures in 2, relapses in 2, improvement in one and death in 8 cases. These cases require larger doses and longer treatment. It is believed that paratracheal adenitis in primary tuberculosis is often a forerunner of meningitis; in these cases the use of streptomycin prophylactically is recommended.—*Streptomycin treatment of tuberculous meningitis in children, K. Choremis, N. Zerros, V. Constantinides & S. Pantazis, Lancet, October 16, 1948, 2: 595.*—(*A. G. Cohen*)

Streptomycin for Meningitis.—Nine patients ranging from 18 months to 35 years of age were treated with streptomycin for tuberculous meningitis. Three of the cases showed a notably good response, although one is blind and the other 2 have not been followed long; the other 6 died. Four of the patients had miliary tuberculosis of the lungs, 2 had chronic pulmonary tuberculosis (one with genito-urinary lesions), one had a tuberculous pleural effusion, and 2 had calcifications in the lungs or spleen. Therapy consisted of intramuscular injections every six hours; the daily dosage ranged from 0.25 Gm in infants to 4 to 8 Gm in adults. Small intrathecal doses were sometimes used. Toxic reactions from the drug were common and often severe. The failure of many brain lesions to respond to streptomycin is probably due to their inaccessibility. These results are similar to those in other reports.—*The treatment of tuberculous meningitis, L. Alperin & J. A. Toomey, J. Pediat., July, 1948 33: 74.*—(*W. H. Oatway, Jr.*).

Tuberculous Mastoiditis.—An infant aged eight weeks was found to have tuberculous mastoiditis. Following a cortical mastoidectomy, streptomycin was administered for fourteen weeks. The treatment resulted in a complete cure. This is noteworthy in view of the previously grave prognosis in such cases; moreover, a less radical surgical procedure was sufficient.—*Tuberculous mastoiditis, O. D. Fisher & E. A. Malkin, Lancet, October 30, 1948, 2: 689.*—(*A. G. Cohen*)

Streptomycin for Pulmonary Tuberculosis.—Good results were obtained in cases of bronchial and laryngeal tuberculosis and tuberculous sinuses of the chest wall. Results were good in cases of tuberculous pneumonia but disappointing in one case of exudative tuberculosis.—*Streptomycin in pulmonary tuberculosis, R. Y. Keers, Lancet, September 18, 1948, 2: 449.*—(*A. G. Cohen*)

Subtilin for Tuberculous Lesions.—Subtilin was administered intraorally by a nebulizer to 8 patients ranging from 18 to 60 years of age. Four of the patients had tuberculous

laryngitis, one had endobronchial disease and the remaining three were considered to have a conspicuous bronchial factor in their pulmonary disease although lesions were not observed in the main stem bronchi at bronchoscopic examination. Subtilin therapy in the latter was directed toward presumed endobronchial lesions in the smaller bronchi. The progress of the disease was followed during therapy by means of frequent physical, fluoroscopic, and roentgenologic examinations. To determine the action and possible toxicity of subtilin, the following laboratory procedures were performed: determination of vital capacity, measurement of daily sputum output, urinalysis, complete blood cell count, sedimentation rate, nonprotein nitrogen and sugar in the blood, protein in the serum, albumin-globulin ratio, icterus index, cephalin flocculation, bromsulfalein liver function test, and phenolsulfonphthalein renal function test. The sensitivity of the tubercle bacilli to the antimicrobial action of subtilin was determined in 6 cases prior to treatment. Of the 4 patients with tuberculous laryngitis, improvement was marked in one, slight in one and questionable in one. In the fourth the disease progressed and the patient died. In the one subject with endobronchial disease, which could be carefully evaluated because of extensive lesions visible at bronchoscopy, it appeared that subtilin favorably influenced the endobronchial disease. After eight and one-half months of therapy bronchoscopy revealed no occlusion or other evidence of endobronchial disease. After ten months of subtilin therapy another bronchoscopic examination revealed inflammation and granulations involving the pharynx, larynx, trachea and bronchi. Tubercle bacilli cultured from the sputum at this time were twice as resistant to subtilin as organisms recovered before treatment. Although the development of marked bacterial resistance was suspected, this was not proved by cultural studies, and the cause of the reactivation of the endobronchial disease is unknown. The condition in the 3 patients in whom endobronchial tuberculosis was presumed to be present in the small bronchi showed no significant change during therapy. Suggestive evidence of the development of bacterial resistance to subtilin *in vivo* was obtained in 3 cases. No evidence of toxicity in the kidneys, liver or bone marrow was encountered. The lack of serious toxicity encountered may be inherent in the mode of administration which results in negligible systemic absorption of subtilin. Administration of subtilin by inhalation was associated with irritation of the respiratory tree in almost all patients during the first two or three weeks, after which the symptoms subsided or disappeared. Asthma developed in one subject and the drug was discontinued. Further investigation of the therapeutic possibilities of subtilin must await basic research directed towards development of derivatives more soluble in physiological fluids. Subtilin in its present form has no place in the treatment of tuberculosis other than for purely investigative purposes.—*Topical application of subtilin to tuberculous lesions in man, S. M. Farber, H. R. Eagle, H. H. Anderson, & R. D. Gorman, J. Lab. and Clin. Med., July, 1948, 33: 799.*—(*F. G. Petrik*)

Prevention of Pulmonary Infection.—Chronic pyogenic bronchopulmonary disease results from primary acute infections of several types. It is essential that the acute conditions be treated vigorously until cured and also that chronic purulent bronchitis be controlled. A study of 50 infants and children who had been treated in the hospital with sulfonamides and/or parenteral penicillin showed many to have had either ill health and progressive pulmonary disease or good health and recurrent respiratory infections in the ensuing year. Pulmonary emphysema and fibrosis often accompanied the chronic lesions. Children with acute or residual infections should be checked at intervals, as are those who have had tuberculosis or rheumatic fever. In those with acute, subacute, or chronic infections, penicillin aerosol therapy may be of value. It may require weeks or months of daily treatments to heal or stabilize the condition and recurrent treatment may be needed. A plastic

nebulizer has been used; a bicycle pump is more convenient and less expensive for prolonged use than oxygen pressure or a hand-bulb. Anti-histaminic drugs alleviate oral sensitivity. Resistant organisms have not developed and sputa have shown only susceptible bacteria. Failure to respond apparently has been due to the serious character of the disease or to an improper therapeutic technique. Best results are obtained in the more acute conditions but hope can be offered the chronic cases. Therapy also removes a public health hazard.—*Prospects for prevention of chronic bronchitis and bronchiectasis, W. Finke, J. Pediat., July, 1948, 33:29.*—(*W. H. Oatway, Jr.*)

Postoperative Pulmonary Complications.—Patients about to undergo abdominal operations were given thorough chest examinations on the basis of which they were graded as good, moderate or bad risks. Alternate cases in each of the groups received 3 inhalation treatments consisting of 100,000 units of nebulised penicillin on the day before operation, and 2 additional treatments on the following day. Bacteriological studies showed considerable diminution in the number of penicillin-sensitive bacteria in the nasopharynx. However, the incidence of postoperative pulmonary complications was not reduced.—*Nebulised penicillin and postoperative pulmonary complications, E. J. Holborrow & E. A. Spriggs, Lancet, October 30, 1948, 2:488.*—(*A. G. Cohen*)

Effect of Medium on M. Tuberculosis.—The effect of Tween 80, bovine albumin, glycerol and glucose on the growth of *M. tuberculosis* H37Rv was evaluated by using nitrogen determinations to determine the amount of growth of tubercle bacilli. The media employed were a modified Proskauer and Beck synthetic medium and a medium recommended by Dubos. The results show that unpurified Tween 80 (0.05 per cent) exerted an inhibitory effect in both media and that purified Tween 80 exerted a similar bacteriostatic effect but to a lesser degree. Both glycerol and glucose markedly stimulate growth. Purified Tween 80 also stimulates growth when added to either of the media in the absence of glucose or glycerol, but not to nearly the same degree as either glucose or glycerol alone. Purified Tween 80 when added to either of the media in the presence of glycerol or glucose inhibited growth as compared with either glucose or glycerol alone. This is in contrast to the findings of Dubos and his collaborators that glycerol inhibited the growth of small inocula in the presence of Tween 80. Bovine serum albumin (fraction V; 0.2 per cent) did not stimulate growth of the tubercle bacillus; it protected the organism against the inhibitory effect of purified and unpurified Tween 80 (0.05 per cent) but only during the first five days of growth. The modified Proskauer and Beck medium containing 2.0 per cent glycerol supported growth of tubercle bacilli at a maximum rate for a longer period than the Dubos medium containing 0.2 per cent glucose.—*The effect of "Tween 80", bovine albumin, glycerol, and glucose on the growth of Mycobacterium tuberculosis var. Hominis (H37Rv), T. H. Sattler & G. P. Youmans, J. Bact., August, 1948, 56: 235.*—(*F. G. Petrik*)

Effect of Tween 80 on Tuberculostasis.—It has been reported that the presence of Tween 80 in synthetic media increased the bacteriostatic action of penicillin, streptomycin, and subtilin on the subsurface growth of tubercle bacilli. Other investigators found, on the contrary, that Tween 80 reduced the inhibitory action of certain compounds on the growth of this organism. As a result of these contradictory reports, this work was undertaken to determine what effect Tween 80 would have on the *in vitro* bacteriostatic activity of 20 compounds for *M. tuberculosis var. hominis* H37Rv. The results show that Tween 80 added to Youman's basal medium without albumin increased the tuberculostatic activity of 15 of 20 compounds, decreased the activity of 3 compounds and had no effect on the action of 2 compounds. The addition of bovine albumin alone to the basal medium reduced

the bacteriostatic action of 13 of the 20 compounds but did not increase the degree of bacteriostasis of any. In the medium with Tween and albumin, 5 compounds were more bacteriostatic than in the basal medium, 4 gave approximately the same results in the two media, and the remaining 11 were less bacteriostatic than in the basal medium. Unpurified and purified Tween 80 gave approximately the same results with 7 of 14 compounds and in the remaining 7 compounds purified Tween 80 produced less inhibition of bacteriostasis than the unpurified detergent. Under the conditions of the experiments the free oleic acid that may be present in Tween 80 was not responsible for the effect of this compound on bacteriostatic activities. In the presence of albumin the bacteriostatic activity of 12 of the 13 compounds was essentially the same with purified and unpurified Tween 80. The explanation for the divergent results obtained with Tween 80 on the 20 compounds employed in this study as compared with the results of other investigators is not apparent. There appears to be no relationship between the action of Tween 80 on these compounds and their chemical structures nor did a fourfold reduction in the concentration of Tween 80 produce significant changes in the results with 5 compounds. The irregular results produced by the presence of Tween 80 on the tuberculostatic activity of compounds indicate the desirability of employing as simple a synthetic medium as possible. Plasma, serum, or albumin can be added to the synthetic medium as a separate test to determine the inactivation of the compound by protein.—*The effect of "Tween 80" in vitro on the bacteriostatic activity of twenty compounds for Mycobacterium tuberculosis, A. S. Youmans & G. P. Youmans, J. Bact., August, 1948, 56: 245.*—(*F. G. Petrik*)

Resistance of Mycobacteria to Streptomycin.—This is a report of studies made of the orderly manner in which spontaneous variants occur in cultures of *M. tuberculosis* and *M. ranae*. The strains of tubercle bacilli used were H37Rv and 2 strains, WS and WR, isolated from the sputa of patients prior to the institution of treatment with streptomycin, one (WS) becoming resistant and the other (WR) remaining sensitive to streptomycin after treatment. Liquid and solid Tween-albumin media were employed. The results show that when large samples of bacteria were seeded on media containing streptomycin, resistant forms were isolated, the number being directly affected by the concentration of the drug in the media. A marked decrease in the number of colonies occurred when the drug concentrations were increased from 1 γ to 10 γ per ml. and then to 100 γ per ml. When certain high concentrations of the drug were used, such as 100 and 1,000 γ per ml., no significant difference in the number of colonies was observed. More resistant variants were found in the strain WS, which later developed resistance to streptomycin after thirty days' treatment, than in WR which remained sensitive to streptomycin. When the sample of bacteria used was sufficiently large and when the time allowed for growth was prolonged, the chances of finding resistant forms were increased. When only one sample is used, the conclusions drawn must be very limited, for great variations were noted among samples from the same parent population. When sensitivity studies are made in the usual manner, a time limit is used for allowing growth of resistant forms. When the time limit is extended, a few resistant forms may be allowed to multiply adequately and the results may be given a quite different interpretation. In addition, the collection of sputum specimens from which the original stock cultures are grown must be considered inadequate in certain instances. All the varitions in resistance of the bacterial population in the diseased areas of the patient are probably never represented in any single or even in several sputum samples. It is conceivable that all the bacteria within a patient are not exposed to the same concentration of streptomycin. Because of the low concentration of streptomycin in certain of the diseased areas, bacteria which may be readily susceptible to streptomycin are allowed to exist during the treatment period and it is con-

ceivable also that an extension of disease in the patient after the treatment period is concluded could be caused by these streptomycin-susceptible organisms. It is essential that complete studies be made of tubercle bacilli obtained from many different areas in patients who have undergone treatment with streptomycin and subsequently have died. It is known that resistance is specific and permanent in nature. Is it not possible that resistant variants may occasionally give rise to mutants resembling the parent strain in their susceptibility to streptomycin? From large populations of a variant of *M. ranae*, which requires streptomycin for growth, a few colonies have been isolated that resemble the parent strain in their susceptibility to streptomycin and sulfonamides, in their biochemical reactions, and in their morphological and cultural characteristics.—*A quantitative analysis of the resistance of Mycobacteria to streptomycin, D. Yegian & R. J. Vanderlinde, J. Bact., August, 1948, 56: 177.—(F. G. Petrik)*

Streptomycin-Resistant Variants.—Streptomycin-resistant variants were obtained from 25 of 57 recently isolated strains of tubercle bacilli. This fact suggests that these variants may play a role in the development of the streptomycin resistance which occurs in cultures of tubercle bacilli isolated from patients who have had prolonged treatment with streptomycin.—*Streptomycin resistant variants obtained from recently isolated cultures of tubercle bacilli, G. P. Youmans & E. H. Williston, Proc. Soc. Exper. Biol. & Med., July, 1948, 68: 458.—(F. B. Seibert)*

Streptomycin and Tuberculosis of Mice.—Streptomycin to the amount of 3,000 γ per day had a favorable effect upon the course of experimental tuberculosis in mice when treatment was initiated seven, fourteen and twenty-one days following infection. All treated mice eventually died and the extent of the pulmonary disease did not differ significantly from the control animals. They all exhibited proliferative lesions, whereas the lesions of the controls were predominantly necrotic exudative. The lesions in the treated animals were extensive and occupied almost all alveoli, but the numbers of tubercle bacilli were considerably reduced as compared with the controls.—*The effect of streptomycin on well established experimental tuberculosis of mice, G. P. Youmans, E. H. Williston, A. S. Youmans, & R. R. Osborne, Proc. Soc. Exper. Biol. & Med., August, 1948, 68: 661.—(F. B. Seibert)*

Dietary Lipids and Experimental Tuberculosis.—Administration of a nonlipid, casein-supplemented ration to resistant Swiss albino mice retarded the progress of experimental tuberculosis. Retardation also occurred on a ration of which 20 per cent was the total fatty acids of cocoanut oil. Olive oil, linseed oil, and oleic acid in a concentration of 20 per cent enhanced the progress of experimental tuberculosis.—*Influence of dietary lipids on experimental tuberculosis, L. W. Hedgecock, Proc. Soc. Exper. Biol. & Med., May, 1948, 68: 106.—(F. B. Seibert)*

Tuberculosis in the Rabbit Ear Chamber.—Microscopic studies of the tissue changes in developing tuberculous infection within a Clark chamber were made. The early reaction to the presence of living tubercle bacilli was minimal. The late reaction was an explosive, necrotizing response. In the one rabbit systematically skin tested with Old Tuberculin this late reaction began coincidentally with the development of skin hypersensitivity. Progressive vascular damage ending in venous thrombosis played an important role in tissue destruction. The bovine tubercle bacillus (Strain Ravenel RV) was used.—*Development of tuberculous infection: In vivo observations in the rabbit ear chamber, R. H. Ebert, J. J. Ahern & R. B. Bloch, Proc. Soc. Exper. Biol. & Med., August, 1948, 68: 625.—(F. B. Seibert)*

Mouse Test for Tuberculosis.—Gastric mucin significantly enhances the virulence of tubercle bacilli for pigmented strains of mice. Grossly visible bacilli are usually present ten days after intraperitoneal inoculation with a dose of about 500,000 bacilli of the H37Rv

strain. Several isolations of tubercle bacilli from patients have been made ten to fifteen days after the inoculation of pigmented mice with mixtures of sputum and mucin. Recently the number of isolations from patients was increased following inoculation of pigmented mice which had been fed the cornmeal-gelatin butter diet of Dubos and Pierce.—*A rapid mouse test for laboratory diagnosis of tuberculosis, A. Milzer & E. R. Levine, Proc. Soc. Exper. Biol. & Med., October, 1948, 69: 16.*—(*F. B. Seibert*)

Tubercle Bacilli in Cerebrospinal Fluid.—In 2 cases of primary tuberculosis in infants, tubercle bacilli were isolated from the cerebrospinal fluid. In neither case was there clinical or chemical evidence of meningitis. One patient was given streptomycin; both patients survived. The source of the bacilli was felt to be a small meningeal lesion which subsequently healed.—*Primary tuberculosis with meningism and bacilli in the spinal fluid, K. Choremis & G. Vrachnos, Lancet, September 11, 1948, 2: 408.*—(*A. G. Cohen*)

Passive Transfer of Hypersensitivity to Tuberculin.—The method of local passive sensitization so extensively employed in human allergy (Prausnitz-Kuster reaction) and in experimental anaphylaxis (Opie and others) can also be applied to the cellular transfer of tuberculin hypersensitivity discovered by Chase. Cells from a peritoneal exudate of a normal guinea pig were injected intracutaneously into the skin of the left flank and cells from a peritoneal exudate of a tuberculous guinea pig were injected into the right flank of a normal guinea pig. After forty-eight hours 0.25 cc. Old Tuberculin with 0.25 cc. saline was given subcutaneously, and fifteen minutes later 1.25 cc. Old Tuberculin with 1.25 cc. saline were given intraperitoneally. Immediate shock occurred followed by complete recovery in a few minutes. Twenty-four hours later a definite reaction occurred in the skin site prepared with "tuberculous" cells but not in the skin of the left flank.—*Passive transfer of local cutaneous hypersensitivity to tuberculin, M. N. Metaxas & M. Metaxas-Bühler, Proc. Soc. Exper. Biol. & Med., October, 1948, 69: 163.*—(*F. B. Seibert*)

Experimental "Wet Lung".—The accumulation of blood, transudates, exudates, or mucus in the lungs following accidental or operative trauma is termed "wet lung" or, loosely, "pulmonary edema." It is a hazard to recovery and the mechanism of production has been poorly understood. A series of experiments has been done to study the problem from the traumatic and neurogenic aspects. Anesthetized dogs were subjected to measured blunt and tearing trauma to the chest wall; "wet lung" was produced. The condition was present not only near the site of injury but often was widespread. The condition could be aggravated by infusions of isotonic saline solution which were harmless in normal animals. Reduction of oxygen intake plus saline infusions also produced "wet lung". Measurement of various blood elements and dynamics did not reveal reasons for the widespread nature of the edema following localized injury. Dorsal sympathectomy afforded marked protection against trauma and infusions, and bilateral cervical vagotomy was also somewhat protective. This suggests that generalized "wetness" is in part a reflex phenomenon.—*"Wet lung"—An experimental study, R. A. Daniel Jr. & W. R. Cate, Jr., Ann. Surg., May, 1948, 127: 830.*—(*W. H. Oatway Jr.*)

Silicosis.—The essential agent in the causation of true or "classical" silicosis is the freshly cloven silica particle. The pathological process basically consists of the hydration of the silica particle at the expense of the cell protoplasm. Because of its atomic lattice structure, quartzite when powdered yields a more virulent dust than other silica formations. Fully hydrated silica (silica hydrosol, sometimes mistakenly called "silicic acid") is nontoxic and enters freely into the metabolism of plants and animals. Partly hydrated suspensions of silica in water ("colloidal silica") retain some of the chemical activity of the dry powder but to a much less degree.—*What is*

silicosis, P. Heffernan, Tubercle, August, 1948, 29: 169.—(A. G. Cohen)

Tuberculosis in Adolescents.—While medical supervision of the school population is good, this is not true of youths after they leave school. The advent of puberty makes these young people peculiarly susceptible to tuberculosis. Three external circumstances combine to add to the hazards of the immediate post-school period: (1) the greater mental and bodily strain of professional life; (2) leaving the parental home for living conditions which may be less favorable; and (3) greater opportunity for infection through wider contact. All forms of childhood and adult tuberculosis are seen in these young people and there is nothing characteristic about the character or the course of the infection. Contrary to common belief, primary tuberculosis heals about as readily in these people as it does in children. Reinfection tuberculosis shows very rapid progression at times, occasionally in the presence of a healing primary focus. The rapid progression from initial focus to advanced disease makes it clear that periodic roentgenographic examination is not the whole answer to early detection, and supervision by the family physician is essential. Two laws are before the legislature that deal with the problem of tuberculosis in adolescents. One authorizes periodic roentgenograms of groups of people at special risk, and the other law deals with compulsory BCG vaccination of all young people who are tuberculin-negative when leaving school. Among 40 cases analyzed, it appeared that 17 were the result of recent primary infection, so that BCG vaccination may presumably be of benefit in somewhat less than half of recognized cases in young adults.—*Die Tuberkulose der Schulentlassenen, M. Wissler & M. Meier, Schweiz. med. Wchnschr., December 27, 1947, 77: 1349.—(M. Marcus)*

Tuberculin Testing of Army Recruits.—Army recruits around the age of twenty were tuberculin tested. Mantoux tests were done at a dilution of 1:1,000 of a solution containing the products of both human and bovine bacilli. The negative reactors were given another test with the same material in 1:100 dilution. It was found that in a group of 1,902 young men, only 20 per cent were negative reactors. The actual figure of negative reactors of this age group is even lower because all patients under treatment were excluded, and because preceding roentgenograms eliminated all those with asymptomatic pulmonary lesions judged to be tuberculous. The figures are in sharp contrast with those obtained elsewhere, notably in France. There a survey conducted in 1946 among army inductees showed that 52 per cent of 100,000 men were tuberculin negative. One may only speculate as to the causes for the discrepancy of results. The author feels that the fact that his antigen also contained products of bovine bacilli may be of significance.—*Premières données des intradermo-réactions tuberculiniques pratiquées chez les recrues suisses, E. Delachaux, Schweiz. med. Wchnschr., December 13, 1947, 77: 1304.—(M. Marcus)*

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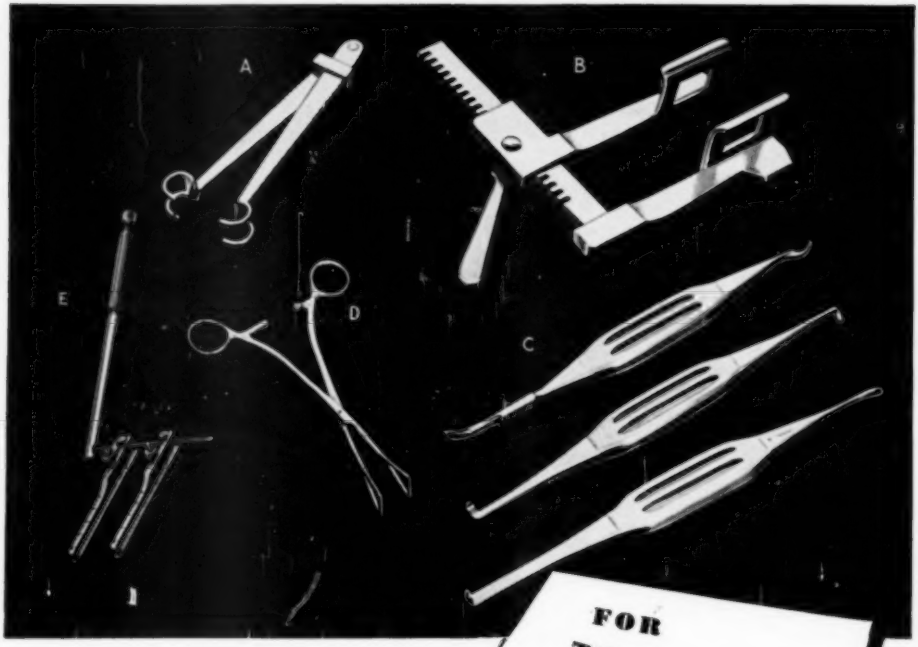

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[1] Rosenthal, J., and Feigin, J.: Some Aspects of the More Common Pathologic Findings in a Hospital for Chronic Diseases, Geriatrics 3:1 (Jan.-Feb.) 1948.

[2] Myers, J. A.: Old Age: The Last Stand of the Tubercle Bacillus, Geriatrics 3:197 (July-Aug.) 1948.

[3] Wiese, E. R.: Tuberculosis in the Aged: Statistical Study, Am. Rev. Tuberc. *41*:210 (Feb.) 1940.

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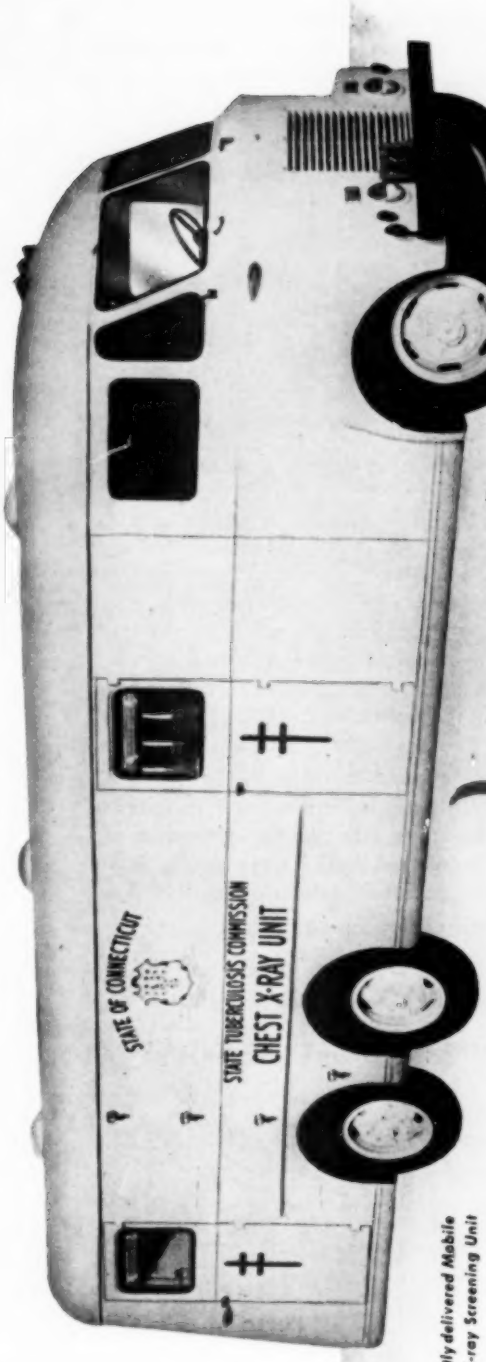

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